THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. XLVI—NO. 3

Issued June 1, 1918

BALTIMORE U. S. A.

1918

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879.

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From two to three volumes each of about six hundred pages are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries $5.25.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 46 JUNE 1, 1918 No. 3

THE REGULATION OF RENAL ACTIVITY

VII. The Balance Between the Regulation by Adrenalin and by Pituitrin

T. ADDIS, A. E. SHEVKY AND G. BEVIER

From the Medical Division of Stanford University Medical School, San Francisco

Received for publication March 18, 1918

Adrenalin (1) and pituitrin (2) have been shown to regulate the excretion of urea in contrary directions. In this paper experiments are given which show that the injection of amounts of adrenalin which

TABLE 1

Comparison of a group of 18 rabbits without and with a mixture of 0.25 cc. of adrenalin and 0.025 cc. pituitrin. No effect on the ratio

PERIOD	WITHOUT MIXTURE			WITH MIXTURE OF 0.25 CC. ADRENALIN AND 0.025 CC. PITUITRIN			PROBABLE DIFFERENCES BETWEEN THE AVERAGE RATIO WITHOUT AND WITH THE MIXTURE OF ADRENALIN AND PITUITRIN	ACTUAL DIFFERENCES BETWEEN THE AVERAGE RATIO WITHOUT AND WITH THE MIXTURE OF ADRENALIN AND PITUITRIN
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio		
	mgm.	*mgm.*		*mgm.*	*mgm.*			
I	43	56	0.76	52	70	0.77	±0.10	+0.01
II	73	62	1.12	72	71	1.16	±0.12	+0.04
III	82	64	1.23	88	65	1.61	±0.14	+0.38
IV	97	68	1.44	87	73	1.50	±0.14	+0.06

greatly increase the activity of the kidney and of amounts of pituitrin which markedly depress that activity, have no effect when they are given together in a certain balanced proportion. On the other hand, when a mixture of both is injected in which this balance is upset by a preponderance of one or the other, a modified adrenalin or pituitrin

Without adrenalin and pituitrin.

With 0.25 cc. adrenalin + 0.025 cc. pituitrin.

Fig. 1. Showing no effect from a balanced mixture of adrenalin and pituitrin.

effect is produced. Adrenalin and pituitrin are thus mutually antagonistic and each may neutralize the effect of the other.

The averages from a group of rabbits under the standard control conditions and after the injection of 0.25 cc. adrenalin alone and of 0.025 cc. pituitrin alone were obtained. A fourth series of experiments was then carried out in which these same quantities of adrenalin and pituitrin were mixed and injected together. The average results of the control and mixture experiments are given in table 1 and figure 1, and the details of the latter in table 5.

TABLE 2

Comparison of a group of 4 rabbits without and with a mixture of 0.25 cc. adrenalin and 0.0125 cc. pituitrin. A modified adrenalin effect on the ratio

PERIOD	WITHOUT MIXTURE			WITH MIXTURE OF 0.25 CC. ADRENALIN AND 0.0125 CC. PITUITRIN		
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	61	73	0.83	28	37	0.75
II	90	74	1.19	63	36	1.83
III	96	74	1.28	55	37	1.54
IV	113	77	1.48	98	39	2.58

As compared with the figures for the controls, those of the adrenalin experiments indicated a marked increase in the urea excreting function and those in the pituitrin experiments, a pronounced decrease. But there is no appreciable change in the mode of action of the kidney when both are given together. The stimulating influence of the adrenalin is almost exactly counter balanced by the depressing effect of the pituitrin.

TABLE 3

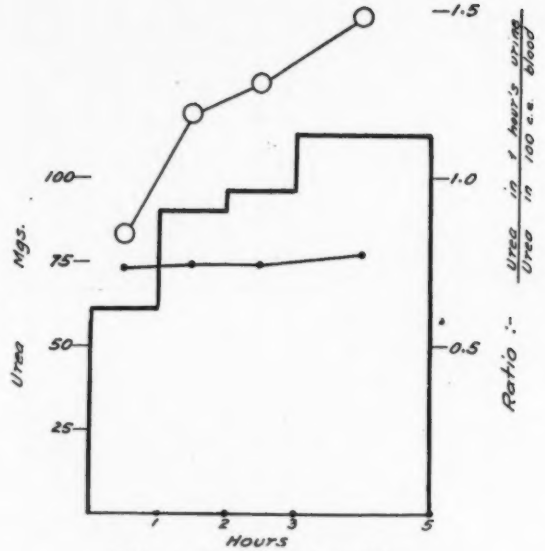
Comparison of a group of 4 rabbits without and with 0.125 cc. adrenalin and 0.125 cc. pituitrin. A modified pituitrin effect on the ratio

PERIOD	WITHOUT MIXTURE			WITH MIXTURE OF 0.125 CC. ADRENALIN AND 0.125 CC. PITUITRIN		
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	61	73	0.83	21	62	0.32
II	90	74	1.19	31	58	0.63
III	96	74	1.28	26	61	0.40
IV	113	77	1.48	37	64	0.52

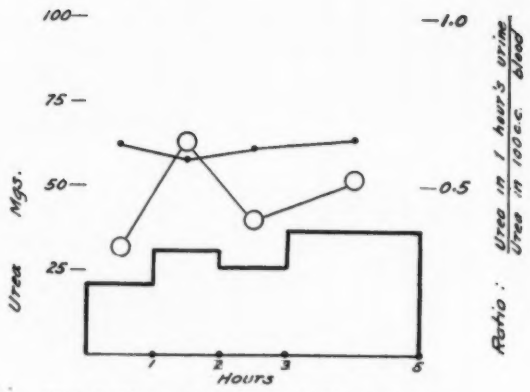
With 0.25 cc. adrenalin + 0.0125 cc. pituitrin.

Without adrenalin and pituitrin.

Fig. 2. A modified adrenalin effect from a mixture of adrenalin and pituitrin in which the adrenalin predominated.



Without adrenalin and pituitrin.



With 0.125 cc. adrenalin + 0.125 cc. pituitrin.

Fig. 3. A modified pituitrin effect from a mixture of adrenalin and pituitrin in which pituitrin predominated.

With a mixture in which the proportion of adrenalin is relatively greater, some increase in the activity of urea excretion is produced though the effect of the pituitrin is still apparent, since the increase is not so great as when the same amount of adrenalin is given alone.

When the proportions are again altered so that the amount of pituitrin is relatively greater than in the balanced mixture, a depression of kidney function follows in which the opposing action of the adrenalin can still be traced.

DISCUSSION

The experiments given in this last paper enlarge the significance of our previous results. For while it is of interest that adrenalin stimulates and that pituitrin depresses the activity of the kidney, the fact that they have effects which are so exactly the reverse of each other that the influence of either may be nullified by the simultaneous administration of a certain quantity of the other, may have a theoretical significance of a higher order. This fact suggests that the resultant of the balance in the blood between the amounts of opposed active principles secreted by the suprarenal and pituitary glands may be a factor in determining the state of renal activity

But there is one aspect of our results from which a deduction of more than hypothetical value may be drawn. Adrenalin and pituitrin, given as we gave them, do not exert their effects on the excretion of urea indirectly through influencing the circulatory conditions in the kidney, but act directly on the urea secreting mechanism. That pituitrin acts through the medium of the nervous system is probable, but with adrenalin there is more than probability, there is a high degree of certainty that it can act only on the termination of sympathetic nerve fibers (3). Since the kidney is supplied with many nerve fibers which end not on the vessels, but on the secreting cells of the glomeruli and tubules, we cannot, in view of the secretory effect of adrenalin, avoid the conclusion that these are secretory nerve endings which it stimulates. And since not only adrenalin, but in all probability pituitrin also, simply accelerate or depress nerve impulses, their effect on the secretory activity of the kidney may be regarded as an argument in favour of a direct regulation and coördination of the activities of the kidney through the central nervous system. We believe that in the case of adrenalin this argument has the force of a proof.

In regard to the hypothesis of an adrenin pituitrin balance in the blood brought to the kidney, we are aware that our experiments only

suggest and do not in themselves prove the existence of such a type of physiological regulation. And the history of theories as to the function of the adrenin of the suprarenal glands based on the results of the intravenous administration of adrenalin is a warning against attaching too much importance to experiments in which active principles are injected from without into the body in ways which can never exactly duplicate the rate and manner in which they are distributed to the tissues from the sites of their formation within the body. Yet we feel that experiments such as ours in which the gland extracts are administered so that they gain entrance to the body only slowly, in minute quantities and over a considerable period of time, should carry more weight in deciding questions of physiological, as opposed to pharmacological, action, than those in which extracts are suddenly injected into the circulation in amounts which, for a short time at least, must induce concentrations of these substances in the blood out of all proportion higher than any which can ever have previously existed there. This latter is a grossly artificial method which certainly bears no relation to physiological processes, while the former is at least more nearly comparable to what we know of the normal modes of entrance of these secretions into the blood stream.

There is one comparatively simple way in which the hypothesis of an adrenin pituitrin balance may be tested. If the suprarenal glands are removed, the pituitary secretion will be left unopposed and there should be a depression of renal activity. Similarly, when the pituitary gland is removed, the suprarenal secretion will be unopposed and the activity of the kidney should be increased.

We had not much more than started this phase of the work when other duties required our attention for an indefinite period of time. We had compared the effect on kidney function of operations in which the suprarenals were exposed, or one suprarenal removed, with the effect of double suprarenalectomy. We found that when both glands were removed, the function of the kidneys was depressed to a much greater degree than after other procedures in which the factors of trauma and anaesthetic were as far as possible the same. The decrease in function was indeed in most cases so marked that little more than traces of urea were excreted and the value of the ratios approached zero. As a consequence the percentage of error of the determination was considerable and these preliminary experiments, though they established the general fact of a great loss of capacity for urea excretion, were not sufficiently accurate to allow us to settle the point in

which we were especially interested, i.e., the form of the ratio-curve in the absence of the suprarenals. We can, however, fully agree with Marshall and Davis' (4) conclusion that the decrease in the rate of excretion of urea, chlorides and creatinin which they observed in cats after double suprarenalectomy may be regarded as evidence of a decrease in the activity of the kidney.

We had not even begun to attempt operations on the pituitary gland. It is stated by Cushing (5) that in dogs the volume of urine is three to five times greater after the removal of the posterior lobe of the pituitary gland, and that this diuresis persisted for some time. It is interesting to note, however, that it was not a permanent condition. In this connection the results of one of our experiments in which a rabbit survived the removal of both suprarenals may be worth quoting. The preliminary control experiment under standard conditions gave an average ratio of 1.54. Five days later both suprarenals were exposed and manipulated through extraperitoneal incisions. Immediately after the animal had recovered from the anaesthetic the experiment was commenced. The average ratio was 2.13, which was exceptional in that such an operation usually reduced the activity of the kidney. One week later the wounds were reopened and both suprarenal glands removed. Ratios were measured immediately afterwards and an average of 0.36 obtained. Though this is a considerable reduction, it is a much higher ratio than we usually obtained after taking out both glands. Next day the rabbit was still alive and the average ratio was found to be 0.68. On the third day after the double suprarenalectomy the ratio had risen to 1.15 and on the seventh day to 1.59. A single experiment is of course of no great value, but it is suggestive when taken in conjunction with the apparently similar experience when the posterior lobe of the pituitary is removed. The simplest explanation, no doubt, is that remaining pituitary or suprarenal tissue, though at first insufficient, was later able to right the balance; but it is also possible that the nervous system may in time accommodate itself to the absence of either pituitrin or of adrenin, so that the equilibrium between those nervous impulses which accelerate and those which depress the activities of the kidney is again reëstablished.

Beyond giving our reasons for concluding that adrenalin and pituitrin, when administered subcutaneously, cannot influence the secretion of urea by virtue of any effect on the circulation of blood through the kidney, we have so far avoided any reference to the rate of blood flow as a factor in determining renal activity. It is, technically, a very

difficult matter to obtain valid data on the relation between blood flow and variations in the secretion of the normal kidney. Stromuhr measurements necessitate a degree of interference with the nervous connections of the kidney which is in itself amply sufficient to cause large deviations from normal blood flow and normal function. But we had hoped we might be able to obtain evidence in regard to this question by a method which does not require the attachment of any mechanical measuring device to the renal vein. By using a syringe with a fine needle it is possible to obtain at intervals samples of blood from the renal vein so that the urea concentration of the blood as it leaves the kidney can be determined. At the same time blood may be taken from the jugular vein as representing the blood of the renal artery, so far as its urea concentration is concerned. If the rate of flow of urine and the rate of urea excretion from the same kidney are measured over the period during which these blood samples are being collected, all the data are at hand for the calculation of the rate of blood flow through the organ. But in rabbits even this relatively minor degree of manipulation was sufficient, in our hands at least, to cause a cessation of the flow of urine. In cats under the influence of powerful diuretics measurements were made, but we cannot place any reliance on them from the point of view of either normal blood flow or normal renal function.

An endeavor was later made to obtain blood from the renal vein so quickly that the decrease in its urea content as compared with that of the general circulation, might be taken as indicating the degree to which the urea excreting activity of the undisturbed kidney had reduced the urea concentration of the blood during its passage through the renal tissues. After a little practice it was found that after a wide and rapid incision through the flank, the left renal vein could be snipped with scissors and a sample of blood collected within a very short space of time, and without touching the kidney. In seventeen of these experiments (6) less urea was found in the blood of the renal vein than in the blood of the renal artery or jugular vein, but the average decrease amounted to only 7.4 per cent of the urea concentration of the general blood stream. Since then the kidney received a much larger amount of urea than it eliminated, it would seem probable that physiological variations in the volume of blood passing through it would have little if any effect on the rate of urea excretion. This argument, of course, is not conclusive since we can never be sure that operative procedures leave kidney function undisturbed, however

quickly they are carried out. Yet it is not negligible when direct evidence is so difficult to secure.

It is an undisputed fact that an extreme reduction in the blood supply to the kidney is followed by a cessation of function. This effect, however, is more probably due to a reduction in oxygen supply than to a decrease in the amount of urinary constituents brought to the kidney. But the oxygen concentration in the blood may be markedly reduced without interfering with renal function. We had commenced observations on the urea secreting capacity under strain in rabbits rendered anemic by the withdrawal of large amounts of blood while the function was being measured. We hope at some future date to be able to continue these experiments, but so far as we went we did not find any significant alteration in the ratio even when the hemoglobin percentage fell below 40 per cent. We believe, therefore, that the kidney is supplied with an excess of both urea and oxygen and that such variations in the total amounts of these substances passing through the kidney as occur with changes in the rate of blood flow through it will not appreciably alter the state of its functional activity, so long as these amounts exceed a certain critical minimum. We regard, then, the total volume of blood supplied to the kidney over any given period as a factor analogous to the total mass of renal tissue (7). Both are ordinarily merely potential factors which become operative as direct determinants of function only under exceptional and extreme conditions.

There remains the question as to the bearing of the theory of a direct regulation of renal secretion through the nervous system and of the subsidiary hypothesis of an adrenin pituitrin balance, on the observed facts in regard to kidney function which were detailed in the first three papers of this series.

Certain of these phenomena—the variation in rates measured at the same blood urea concentration, the increase in the activity of the kidney under increasing strain and the decrease in variability with increase in strain—were observed in man as well as in the rabbit. No discussion is required to show that they are just such phenomena as might be expected to occur in the function of any organ under the influence of the nervous system. Their relation to the hypothesis of an adrenin pituitrin balance in the blood has been dealt with elsewhere (8).

But there are still two characteristics of normal kidney function, one peculiar to the rabbit—the gradual increase in the urea excreting capacity during successive observations (9)—and the other the rela-

tionship between water administration and states of activity in urea excretion, which we have noted but have not hitherto attempted to explain.

There was nothing in the observations we carried out on man to lead us to expect any increase in the rate of urea excretion in consecutive observations on rabbits whose blood concentration remained constant, and no explanation suggested itself until we had commenced the work with adrenalin. It then occurred to us that a difference between the conditions of our experiments on man and on the rabbit may have been responsible for this striking divergence in the behavior of the kidney. In man there was no catheterization and by using sharp needles even minor degrees of discomfort were avoided in obtaining the blood. The subjects were instructors and students who were in no anxiety at the prospect of being bled. In the rabbits, on the other hand, there was a series of nine manipulations over a period of five hours commencing with the passage of the stomach tube, against which they usually struggled violently, and often involving, in the catheterizations, a certain amount of trauma on account of the compression over the bladder and the repeated rotations and partial withdrawals and reintroductions of the catheter, employed in the endeavor to make sure that the urine or wash water had been removed as completely as possible. These differences in the external conditions in the experiments on man and on the rabbit induced a difference in the internal conditions also, for while in the one case the subjects were undisturbed, in the other they were much excited.

The influence of excitement on the rate of secretion of adrenalin is still under discussion, for Stewart and Rogoff (10) have not found the increase which Cannon and others (11) observed. But some recent measurements of Bedford (12) happen to parallel closely the time interval relations of our experiments. He produced shock in anaesthetized animals and determined the concentration of adrenalin in the blood of the suprarenal vein in successive samples drawn at intervals over a period of several hours. It was found that the concentration of adrenalin did not at once rise to a high level but only gradually increased so that the maximum was not reached for several hours. The increase, though slow, was pronounced. Thus in one case there was over thirty times more adrenalin at the end than at the commencement of the experiment. Now it will be remembered that the increase in the activity of the kidney in our experiments is also gradual and does not approach its highest point until after three or four hours.

When this concordance between the time required for the suprarenal glands to markedly increase their adrenin putput and the time at which the rabbit's kidney shows its greatest activity during a period of continued excitement, is taken in conjunction with the fact that the subcutaneous injection of adrenalin accentuates the degree of increase in activity but does not otherwise alter the mode of action of the kidney, there seems to be reason for the supposition that this increased activity of the kidney of the rabbit under these conditions is associated with and is intensified by an increase in the adrenin content of the blood arising under the influence of the physiological stimulus of excitement.

The only other observation whose bearing on our hypothesis remains to be discussed, is the relation between water administration and renal activity in the excretion of urea. We have not given any details of the influence of adrenalin and of pituitrin on the excretion of water, partly because it has been already described by others, (13), (14), but mainly because we have not the data which are necessary to decide whether or not the changes they induce in water excretion arise from alterations in renal activity. It is evident that the mere observation that the output of a urinary constituent is altered by adrenalin and pituitrin does not in itself prove that any change in the activity of the kidney has occurred. For the kidney may have maintained its accustomed mode of reaction and the altered output be the result of its passive adjustment to changes in the concentration of that constituent in the blood. It is only for the urea excreting function that we have excluded this possibility. Yet we believe it is highly probable that it will be found that all the various activities of the kidney are stimulated by adrenalin and depressed by pituitrin and that the increase in the volume of urine after adrenalin and its decrease after pituitrin is, in the main at least, caused by alterations in renal activity and not by changes in the water content of the blood. We refer to this question now because in studying the effect of the administration of varying amounts of water on urea excretion, we observed a curious divergence in its influence under different conditions.

Our first experiments on the effect of water were carried out with a view to determining whether the changed action of the kidney in excreting urea after adrenalin and pituitrin might not be a secondary result of the concomitant change in urine volume. But when we gave 100 cc. of water so that the volume of urine was increased to a degree approximating that obtained after adrenalin, there was no increase in the urea excreting function nor any appreciable decrease when the

volume of urine was lowered to amounts equivalent to those obtained after pituitrin by the administration of magnesium sulphate by mouth. This early result indicated that urea and water effects were independent and forecast our later conclusion that there was no causal relation between variations in urine volume and variations in renal capacity for urea excretion. In this particular instance, therefore, in which moderate quantities of water were given, there was no accompanying change in the activity of the kidney for urea excretion.

We have elsewhere (15) referred to other experiments, carried out on man, in which the drinking of very large quantities of water was associated with a constant acceleration of the work of the kidney in excreting urea. In these experiments the subject drank 1000 cc. of water and thereafter every quarter or half hour amounts of water equivalent to the volume of urine excreted. In this way the water excreted was continually returned so that the water content of the body was for a period of several hours kept much higher than it normally would have been. The rate of water excretion gradually increased until enormous quantities were being eliminated, amounting in some cases to a 24 hour rate of over 20,000 cc. Under these conditions, there was a very definite increase in the rate of urea excretion, though there was no significant change in the level of the blood urea concentration. We therefore concluded that in this instance, in which very large quantities of water had been given, some unknown factor had produced an increase in the urea excreting activity of the kidney. The nature of this factor was left undetermined but we decided it was not the increased water content of the blood because there was no constant relation between the amounts of water eliminated and the degree of increase over the normal in the rate of urea excretion.

The third instance in which the giving of water was accompanied by the reverse effect, i.e., by a decrease in urea excreting activity, was found in some experiments on rabbits. A double set of seventeen observations were made on a group of ten rabbits, with and without the administration of 25 cc. of water by stomach tube. The averages are given in table 4 and charted in figure 4.

We were at first glance inclined to think that the decrease in the ratios in the water experiments was due to chance, for the actual differences are not great. But they are in the same direction in each period and calculation from the probable differences of the averages of the ratios shows that there is only about one chance in one hundred and seventy that this was a chance variation. We were therefore

TABLE 4

Comparison of averages from 17 experiments on a group of 10 rabbits without and with 25 cc. H_2O

PERIOD	WITHOUT WATER			WITH 25 CC. WATER			PROBABLE DIFFERENCES BETWEEN THE AVERAGE RATIOS WITHOUT AND WITH WATER	ACTUAL DIFFERENCES BETWEEN THE AVERAGE RATIOS WITHOUT AND WITH WATER
	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio	Urea in one hour's urine	Urea in 100 cc. of blood	Ratio		
	mgm.	*mgm.*		*mgm.*	*mgm.*			
I	48	60	0.76	43	64	0.65	±0.068	−0.11
II	67	62	1.14	72	70	1.00	±0.075	−0.14
III	82	63	1.29	84	71	1.16	±0.097	−0.13
IV	100	69	1.55	95	71	1.34	±0.106	−0.21

TABLE 5

A mixture of 0.25 cc. adrenalin and 0.025 cc. pituitrin

Rabbit No.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in urine	Urea in blood	Ratio	Urea in urine	Urea in blood	Ratio	Urea in urine	Urea in blood	Ratio	Urea in urine	Urea in blood	Ratio
85	3	38	0.00	13	38	0.35	29	44	0.67	55	40	1.38
86	lost	lost	lost	31	42	0.73	75	42	1.78	73	43	1.69
88	43	72	0.59	82	72	1.14	88	73	1.21	127	75	1.69
96	84	66	1.27	104	66	1.57	133	69	1.93	132	65	2.02
97	86	51	1.69	93	48	1.93	94	45	2.08	92	47	1.95
98	28	39	0.70	46	39	1.19	99	33	3.00	76	32	2.38
100	2	105	0.00	8	111	0.07	7	113	0.06	5	118	0.05
93	58	58	1.21	74	45	1.63	99	45	2.20	109	51	2.14
65	14	34	0.26	67	54	1.24	91	56	1.64	89	54	1.64
66	9	32	0.28	33	39	0.85	25	33	0.77	12	60	0.20
67	17	54	0.31	36	56	0.64	33	56	0.58	61	60	1.01
68	75	54	1.38	104	60	1.74	106	68	1.56	116	72	1.61
71	27	54	0.50	50	54	0.93	66	57	1.16	64	62	1.03
73	55	57	0.96	128	46	2.78	119	50	2.38	75	53	1.41
90	16	21	0.77	12	19	0.62	63	24	2.63	88	26	3.39
99	62	72	0.86	93	97	0.96	104	69	1.50	104	78	3.33
103	157	154	1.02	123	144	0.85	197	138	1.43	139	145	0.96
105	85	134	0.64	117	153	0.76	lost	lost	lost	94	149	0.63
Average.....	52	70	0.77	72	71	1.16	88	65	1.61	87	73	1.50

Without water. With 25 cc. water.

Fig. 4. Showing a slight depression of renal activity following the administration of water.

forced to conclude that the giving of relatively small amounts of water to rabbits was accompanied by a decrease in the urea excreting activity of the kidney due to the operation of some unknown factor. In our view the reason for both the increase in kidney activity after water administration in man and the decrease which occurred in the experiments on the rabbit is to be found in a consideration of the divergent effect of the water, not on the kidney alone but on the body as a whole.

In the experiments on man we continually nullified the work of the kidney by pouring back the water which it had eliminated. We thus tended to produce an artificial enrichment of the organism with water and the kidney made greater and ever greater efforts in preventing a harmful disturbance in the osmotic equilibrium of the tissues. We believe that the diuresis which occurred was greater than could be accounted for mechanically through the increase in the free water of the blood, and that the unknown factor which stimulated the kidney to these more than wonted exertions was the accelerating influence of nerve impulses aided by an increase in the relative proportion of the adrenin as compared with the pituitrin of the blood. The indication of the operation of this factor is the increased activity in the excretion of urea.

In the experiments on the rabbit no excessive amount of water was given. A quantity of 25 cc. no doubt seems a large amount for a rabbit, and in proportion to the body weight may be regarded as equivalent to about 750 cc. for a man. But quantities are large or small only in relation to the requirements of the tissues, and in these rabbits the need of the body for water was great since they had received no food or water for at least seventeen hours before the experiment began. But this water introduced into the gastro-intestinal tract could only reach the tissues through the blood stream and in doing so would increase the concentration of free water in the blood. The unregulated kidney must automatically respond to this stimulus and an undue proportion of the needed water would have been lost to the body. We believe, therefore, that when food and water are withheld there is a purposeful adaption of kidney function through a relative increase in the pituitrin as compared with the adrenin content of the blood, and that the decrease in the activity of the urea excreting function is only one example of a general state existing in all the departments of renal function. This regulation is, of course, not peculiar to the rabbit. We have elsewhere cited instances of the decrease in function in man which occurs after periods of abstention from food and water (16).

These explanations of our results have only a hypothetical value. For we have not demonstrated any change in the adrenin pituitrin balance, and we have only assumed that the excretion of water resembles the excretion of urea in varying in a manner which cannot be fully accounted for by changes in the water content of the blood. Until some method is devised by which the adrenin and pituitrin content of the blood reaching the kidney can be determined, and until the water in the blood available for excretion can be measured, these assumptions must remain untested by experiment

The question of the exact mechanism whereby the kidney is regulated is therefore not definitely decided by these experiments. We should indeed be quite prepared to find that changes in the adrenin-pituitrin balance are only operative in times of emergency as adjuvants to the direct nervous control. The fact that all the nerves going to the kidney may be cut and that the kidney may even be transplanted on to the splenic vessels and yet continue to function "normally" (17) does not exclude this possibility. Our criterions of normality are still too vague.

The question which *is* decided is that there is a regulation in addition to, and distinct from, the mechanical regulation arising from the passive adjustment of the kidney to changes in the concentration of urinary constituents in the blood. And still more important is the demonstration that this higher regulation may annul or overrule the influence of physical and chemical factors in the environment of the kidney, in order that the requirements of the body as a whole may be met. The kidney can not be regarded as an isolated mechanism. It is coördinated in those active adaptions which continuously maintain the harmonious equilibrium of the living organism.

CONCLUSIONS

1. The subcutaneous injection of amounts of adrenalin which increase the urea excreting activity of the kidney, and of amounts of pituitrin which depress that activity, have no effect when they are injected together in a certain balanced proportion.

2. All grades of stimulation or depression may be induced by the injection of mixtures of adrenalin and pituitrin in which this balance is deflected by a preponderance of one or the other.

3. In the rabbit the removal of both suprarenal glands is followed by a depression of the urea excreting activity of the kidney, which is

greater than that which follows similar operations in which the suprarenals are not removed.

4. The facts in regard to urea excretion given in these and other papers are reviewed and from them the conclusion is reached that under physiological conditions the urea excreting activity of the kidney is determined by two main factors. There is a fixed and mechanical regulation through the urea concentration of the blood but there is also another and overruling type of regulation which acts through the medium of the central nervous system.

5. The mode of action of the regulation through the nervous system is discussed and it is suggested that variations in the balance between the rates of secretion of active principles from the suprarenal and pituitary glands may play a part in the mechanism through which it acts.

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THE EFFECT OF HOLDING THE BREATH AND OF REBREATHING ON THE RISE OF CO_2 TENSION IN THE LUNGS, AND THE DETERMINATION OF THE CO_2 TENSION OF THE "VENOUS PULMONARY AIR"

HENRY LAURENS

From the Physiological Laboratory, Yale School of Medicine

Received for publication March 23, 1918

INTRODUCTION

A method has been recently suggested by Henderson and Prince (1) for the determination of the CO_2 tension of the "venous pulmonary air" and attention called to the importance of this determination. Christiansen, Douglas and Haldane (2) have also described a method for the determination of this function based on the use of the lungs as an aerotonometer. They found that the CO_2 pressure in the mixed venous air could not be obtained by holding the breath for the reason that the alveolar CO_2 pressure continues to rise with the length of time that the breath is held, there being no pause to indicate when the CO_2 in the alveolar air is in equilibrium with that of the venous blood. Their method is briefly as follows: After a maximal expiration a maximal inspiration of a mixture of CO_2 and air is made and the gas mixture held in the lungs for a few seconds, and then exhaled and an alveolar sample analyzed for CO_2. After a further interval another, and sometimes a third, alveolar sample is taken and analyzed. From the increase or decrease of CO_2 in the mixture after inhalation the venous CO_2 pressure is inferred, after correction for the influence of oxygenation in the lungs.

Boothby and Sandiford (3) also used the aerotonometric method to determine the CO_2 tension in the venous blood. Their method is a modified form of that of Christiansen, Douglas and Haldane. After a mixture of CO_2, air and O_2 is inspired, it is held for about five seconds and then an expiration to the mid-level made for the first alveolar sample, after which the breath is held for as long as possible and a maximal expiration made and the second alveolar sample taken.

In an earlier paper Boothby (4) calculated the venous CO_2 tension by the nitrous oxide method, from the consumption of O_2, the flow of blood through the lungs, the respiratory quotient and the percentage saturation of the Hb.

Wardlaw (5) has recently studied in detail the rise in CO_2 tension of the air in the lungs when the breath was held for various periods of time and when the air was rebreathed from a bag. In the case of holding the breath he found that as the length of time increased the alveolar CO_2 tension rose at a continually decreasing rate for about 30 seconds, the alveolar tension being an exponential function of the period for which the breath was held, and that when the breath was held for a long enough period the CO_2 tension gave indications of attaining a certain fixed value, (48.5 mm. Hg.).

When the same air was rebreathed Wardlaw found again that the alveolar CO_2 tension rose at a continually decreasing rate, reaching a final value of about 56.5 mm. Hg. During the first five seconds the CO_2 tensions rose by practically the same amount as when the breath was held, but between the 25th and 30th seconds the rise was about three times as great as when the breath was held. Furthermore, the total rise in the alveolar CO_2 tension in 35 seconds was nearly 40 per cent greater than the rise occurring in the same period when the breath was simply held, the alveolar tension being again an exponential function of the period for which the contents of the lungs are rebreathed.

The rate of gaseous exchange in the alveolar air is therefore about twice as great when the movements of breathing are performed as when the breath is held under normal pressure. The cause of this greater increase Wardlaw does not believe to be due to a slowing up of the circulation during the time that the breath is held, for by comparing the effect on the gaseous exchange of one respiratory movement in 20 seconds and of three in the same period with that occurring when the breath is held, he found that the increase in CO_2 was the same whether one or three movements were made. Also he was led to conclude that the increase in the respiratory exchange in a given time was independent of the extent of the respiratory movements; for he again obtained the same increase when four respiratory efforts with the pharynx closed were made as when one or three normal respiratory movements were made in the same period.

Wardlaw also concluded from experimental evidence that holding the breath under increased pressure does not affect the gaseous ex-

change. On the other hand, holding the breath under negative pressures he found to increase it to such an extent that when the breath was held under pressures of more than −10 mm. Hg. the same acceleration in the rate of the gaseous exchange took place as when the air was rebreathed from a closed bag. He therefore concludes that the respiratory exchange is accelerated during breathing owing to the existence of negative pressure in the chest during the act of inspiration.

It is difficult to reconcile this conclusion with the results of the comparison of the rate of gaseous exchange made by Wardlaw when one and three respiratory movements are made in 20 seconds; for, if by one respiratory movement is meant an inspiration lasting for 20 seconds (which is improbable) then, if the increase in the gaseous exchange is due to the negative pressure, the CO_2 tension should be greater than when three respiratory movements are made in 20 seconds, since the time during which a negative pressure exists is shorter in the latter case. If by one respiratory movement in 20 seconds is meant an inspiration lasting 10 seconds and an expiration lasting 10 seconds, than a negative pressure will exist for an equal length of time in the two cases. But if by one respiratory movement is meant an inspiration of usual duration, followed, after 20 seconds, during which time the breath is simply held, by an expiration, than a negative pressure would exist in the lungs a shorter length of time than when three respiratory movements are made in the same period. This would also be true when the respiratory movements were made with the pharynx closed, since during the time that the expiratory efforts were being made the pressure would be positive, and therefore the rate of exchange would be slowed up, as compared with the rate during the time that the pressure was negative, that is, during the actual inspiration.

THE RISE OF CO_2 TENSION IN THE LUNGS IN HELD AND REBREATHED AIR

In connection with the study of the circulation rate it was suggested to me by Prof. Yandell Henderson that it would be well to make a detailed study of the changes in the CO_2 tension of held and rebreathed air along the lines followed by Wardlaw, and to check up the determination of the venous CO_2 tension by the method suggested by Henderson and Prince.

This most simple method of determining the venous CO_2 tension consists briefly in intermittent, as contrasted with continuous, rebreathing. After a sudden inspiration a quick and deep expiration is made

into a rubber bag and the CO_2 tension of this "mixed air" determined by analysis. After a sufficiently long interval to allow the respiration and circulation to return to normal, the lungs are emptied as far as possible and the contents of the bag inhaled, the breath held for a few seconds and the air then exhaled deeply into the bag and its CO_2 tension again determined. This intermittent rebreathing is continued until after successive rebreathings a constant CO_2 tension is found upon analysis. This takes place usually, when the subject is at rest, after the fifth to the sixth inhalation.

In all of the experiments which are described below, on holding the breath and rebreathing air this method of Henderson and Prince was employed to obtain the first gas mixture wherewith to begin. In this way an experimental starting point was obtained in that, by analysis of the expired air, a known CO_2 tension was begun with. Also the percentage of increase can then be calculated. The CO_2 tension of the air obtained in this way was found to vary between 3.25 and 4 per cent.

The greater increase described by Wardlaw in CO_2 tension when air is rebreathed as compared with that obtained when air is held in the lungs has been corroborated. The increase in the CO_2 tension when the breath is held is shown in table 1 and figure 1. The tension of the CO_2 continues to increase with the length of time that the breath is held, but at a decreasing rate, until an apparent maximum is reached as indicated by the percentage of increase. It is impossible for me to hold my breath, under the conditions of the experiment, for longer than about 50 seconds.

Results obtained when air is rebreathed are shown in table 2 and figure 1. Here again the CO_2 pressure continues to rise at a decreasing rate with the length of time that the air is rebreathed and attains also an apparent maximum. The rate of increase, however, is more rapid and the final tension attained higher when the air is rebreathed than when it is held. It is impossible for me to continue rebreathing, under the conditions of the experiment, for longer than 72 seconds, (12 rebreathings). The maximum CO_2 tension seems to be approximately attained after the 9th rebreathing (54 seconds).

This comparison therefore between the rate of CO_2 tension increase and the final tension attained after holding the breath and rebreathing the same air, gives results which are essentially similar to those of Wardlaw. When the air is rebreathed the tension of CO_2 rises more rapidly and attains a higher value than when the breath is held. After

30 seconds for example, there is an average percentage increase of 178 when the air is rebreathed as compared with one of 162 when the breath is merely held.

TABLE 1

The influence of holding the breath on the CO_2 tension

MINUTES HELD	PERCENTAGE OF CO_2								
	Before holding	After holding	Per cent of increase	Before holding	After holding	Per cent of increase	Before holding	After holding	Per cent of increase
5	3.31	4.07	123	3.65	4.34	119	3.47	4.48	129
10	3.43	4.72	138	3.42	4.55	133	3.56	4.98	140
15	3.51	5.33	149	3.54	4.78	135	3.36	4.91	146
20	3.50	5.39	154	3.45	5.14	149	3.61	5.67	157
25	3.25	5.10	157	3.43	5.45	159	3.65	5.95	163
30	3.34	5.34	160	3.52	5.74	163	3.54	5.79	164
35	3.49	5.72	164	3.53	5.79	164	3.67	6.13	167
40	3.57	5.96	167	3.58	6.05	169	3.42	5.95	174
45	3.70	6.40	173	3.58	6.12	171	3.35	5.80	173
50	3.77	6.41	170	3.67	6.39	174	3.49	6.11	175

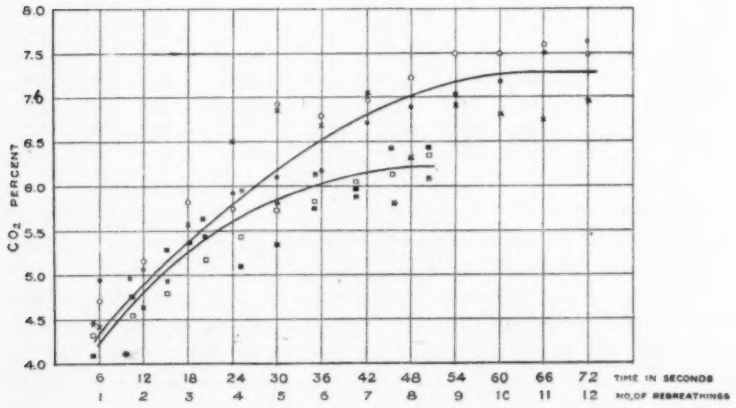

Fig. 1. The influence of holding the breath (lower curve) and of rebreathing (upper curve) on the rise of CO_2 tension in the lungs.

A series of experiments, along the same lines that Wardlaw followed was next made to determine the cause of the greater and more rapid increase in CO_2 tension when the air is rebreathed than when it is

held. This involved in the first place the investigation of the effect of varying the number of respirations in unit time.

Table 3 shows the results obtained for 10, 20 and 30 seconds. In general it may be said that increasing the number of respirations in unit time increases the tension of CO_2 in the rebreathed air. In the case of 10 seconds this fact does not come out so clearly. But another significant fact, which will be referred to later, (see tables 6 and 7), is noted; this is that holding the breath for 10 seconds and inspiring it for 5 seconds and expiring it for 5 seconds results in about the same

TABLE 2

The influence of rebreathing on the CO_2 tension

MINUTES REBREATHED AND NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2								
	Before	After	Per cent of increase	Before	After	Per cent of increase	Before	After	Per cent of increase
6 (1)	3.69	4.94	134	3.67	4.66	127	3.40	4.45	131
12 (2)	3.59	5.10	142	3.51	5.19	148	3.31	4.63	140
18 (3)	3.35	5.39	161	3.69	5.83	158	3.52	5.63	160
24 (4)	3.39	5.90	174	3.50	5.74	164	3.86	6.52	169
30 (5)	3.40	6.12	180	3.86	6.87	178	3.93	6.88	175
36 (6)	3.30	6.14	186	3.64	6.55	180	3.75	6.71	179
42 (7)	3.45	6.73	195	3.78	6.99	185	3.86	7.03	182
48 (8)	3.50	6.90	197	3.62	7.17	198	3.46	6.32	185
54 (9)	3.41	7.02	206	3.77	7.50	199	3.67	6.94	189
60 (10)	3.57	7.21	202	3.75	7.50	200	3.54	6.83	193
66 (11)	3.56	7.55	212	3.71	7.61	205	3.49	6.75	194
72 (12)	3.69	7.68	208	3.67	7.52	205	3.63	6.97	192

percentage increase of CO_2. This is also seen in the case of 20 seconds, where inspiring for 10 seconds and expiring for 10 seconds results in about the same increase as holding the breath for 20 seconds.

Wardlaw obtained the same percentage of CO_2 increase over that found after holding the breath, when one respiratory movement in 20 seconds was made as when three were made. My results do not agree with this. In the first place, one respiratory movement (in 10 or 20 seconds) in which the inspiratory and expiratory portion each have a duration of one-half of the whole period, results in a CO_2 increase which is about the same as when the breath is simply held for the whole period. And in the second place, the number of respirations does influence the percentage of CO_2 as seen by the percentage increase when two or more movements are made.

TABLE 3

The effect of varying the number of respiratory movements in unit time on the rise of CO_2 tension

10 seconds

HOLDING BREATH		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 1 MOVEMENT		INSPIRATION 2½ SECONDS EXPIRATION 2½ SECONDS 2 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.69–5.11	138	3.62–4.96	137	3.44–4.87	142
3.55–4.79	135	3.55–4.81	136	3.57–5.06	142
3.60–4.88	136	3.58–4.83	135	3.66–5.11	140
3.46–4.81	139	3.58–4.93	137	3.53–5.12	145
3.37–4.55	135	3.70–4.81	130	3.39–4.81	142
				3.42–4.83	141
*Average = 137		Average = 135		Average = 142	

20 seconds

HOLDING BREATH		INSPIRATION 10 SECONDS EXPIRATION 10 SECONDS 1 MOVEMENT		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 2 MOVEMENTS		INSPIRATION 3½ SECONDS EXPIRATION 3½ SECONDS 3 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.84–5.80	151	3.77–5.47	145	3.52–5.60	159	3.55–5.94	167
3.62–5.67	157	3.81–5.65	148	3.49–5.71	163	3.56–6.04	169
3.52–5.48	156	3.71–5.79	156	3.61–5.87	163	3.60–6.08	169
3.51–5.51	157	3.51–5.37	153	3.66–5.86	160	3.67–6.06	165
		3.59–5.67	158	3.48–5.72	164		
		3.63–5.55	153				
		3.34–5.28	158				
Average = 155		Average = 153		Average = 162		Average = 168	

30 seconds

HOLDING BREATH		INSPIRATION 7½ SECONDS EXPIRATION 7½ SECONDS 2 MOVEMENTS		INSPIRATION 3¾ SECONDS EXPIRATION 3¾ SECONDS 4 MOVEMENTS		INSPIRATION 3 SECONDS EXPIRATION 3 SECONDS 5 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.72–5.77	155	3.71–6.20	167	3.50–6.19	177	3.40–6.12	180
3.58–5.52	154	3.46–6.03	174	3.64–6.27	172	3.86–6.87	178
3.38–5.49	162	3.55–6.20	175	3.77–6.45	173	3.93–6.88	175
3.61–6.01	166	3.41–5.76	169	3.71–6.42	173		
3.40–5.44	160	3.72–5.90	156	3.32–6.04	182		
3.56–5.74	161	3.61–6.01	166	3.62–6.37	173		
Average = 160		Average = 168		Average = 175		Average = 177	

The explanation of the approximate same percentage of increase of CO_2 when the air is inspired for 5 or 10 seconds and then expired for an equal length of time, as when the breath is held for the whole time, is probably due to the longer duration of the negative pressure during the inspiration, which results in accelerating the rate of the CO_2 exchange. Probably also the blood stream is retarded while the breath is held and therefore less CO_2 is exchanged during the period although it is a longer time. It would seem that the CO_2 exchange takes place for the greater part during the actual inspiration of the air, that is, during the time that there is a negative intra-pulmonic pressure which, when the breath is quickly inspired and then held, is relatively short,—since the negative intra-pulmonic pressure quickly rises to atmospheric after inspiration—as compared with that existing when the actual act of inspiring the air lasts for 5 or 10 seconds.

The hindrance to the circulation during the period that the breath is held must play an important part, although Wardlaw does not admit that it does. Hill and Flack (6) also found a lower tension of CO_2 after holding the breath for a certain period than when the air was rebreathed. They explain this as being due to a mechanical obstruction of the circulation. Douglas and Haldane (7) also think that there is a slight circulatory block when the breathing is suspended. The findings of Ebert (8), contrary to the opinion of Wardlaw, do not seem to me to bear on the particular point of the retarded circulation during the time the breath is held. Ebert found that the flow of blood through the lungs was hastened during inspiration and retarded during expiration.

If the greater increase in carbon dioxide tension with the number of respiratory movements is due to the negative intra-pulmonic pressures during the actual act of inspiration as Wardlaw claims, then his results on varying the frequency of the respiratory movements and which he found not to affect the increase in the percentage of carbon dioxide are incomprehensible. For certainly the negative pressure exerted by the inspiratory portion of the three respiratory movements must be approximately three times as effective as that exerted by one respiratory movement. My results show that the frequency of respiratory movement does affect the velocity of the carbon dioxide exchange in the lungs so that, whatever its cause, it must act every time a respiratory movement is made.

The next series of experiments consisted in investigating the effect of varying the amplitude of the respiratory movements on the relative

percentage of CO_2 in the rebreathed air. The percentage of CO_2 when the breath was held was compared with that found when it was freely rebreathed, and with that found when inspiratory and expiratory efforts were made with the glottis closed.

Wardlaw found by this method that the CO_2 of the alveolar air had the same tension when four respiratory efforts with the glottis closed were made in 20 seconds as when one or three normal respiratory movements were made. My results (see table 4) again differ in some particulars with his. In my experiments the air in the bag, after its CO_2 tension had been determined by analysis, was inhaled quickly and then, with the glottis closed, inspiratory and expiratory efforts were made, each for the stated number of seconds and for the stated number of times, and then the air quickly exhaled into the bag and its CO_2 percentage again determined.

Rebreathing the air, or performing inspiratory and expiratory movements with the glottis closed, results in about the same increase in CO_2—a little higher, probably due to the air being in the lungs and air passages all the time—over that obtained when the breath is held, as free rebreathing does (Cf. tables 3 and 4).

The number of respiratory movements in unit time thus influences the CO_2 tension when the glottis is closed as it does when it is open. The conclusion therefore seems obvious that the respiratory movements, or rather the inspiratory portions, hasten the rate of gaseous exchange.

When an act of inspiration or of expiration is made with the glottis closed the pressure in the lungs falls and rises with the rarefaction or compression of the contained air. Also at each inspiration there is, due to the increased negativity of intrathoracic pressure, an increase in the aspiratory action and an increase in the venous flow to the right heart, and consequently more blood thrown into the pulmonary circulation. In rebreathing into and out of a bag, the increase in the pulmonary circulation during inspiration must increase the velocity of the gaseous exchange. During expiration the decreased flow of blood and the consequent retarding of the velocity of the gaseous exchange do not play an important part in the comparative amount of CO_2 exchanged during rebreathing and when the breath is held, either when the air is kept in the lungs by closing the glottis during an expiratory movement or when the air is blown out into the bag. With the next inspiratory and expiratory movement the process is repeated. We would therefore expect when respiratory movements are made

with the glottis closed, just as when the movements of free rebreathing are performed back and forth into a bag, that the percentage of CO_2 in the air would be greater than when the breath is simply held, because of the increasing effect upon the velocity of the gaseous exchange of the increased circulation or of the negative intra-pulmonic pressure

TABLE 4

The effect of varying the depth of the respiratory movements on the rise of CO_2 tension

10 seconds

HOLDING BREATH		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 1 MOVEMENT		INSPIRATION 2½ SECONDS EXPIRATION 2½ SECONDS 2 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.48–4.91	141	3.38–4.85	143	3.63–5.41	149
3.33–4.33	130	3.44–4.98	145	3.42–4.86	142
3.34–4.58	137	3.27–4.55	139	3.53–5.06	151
3.80–5.18	136			3.50–4.97	151
3.68–5.08	138			3.67–5.25	143
3.50–5.01	143				
Average = 138		Average = 142		Average = 146	

20 seconds

HOLDING BREATH		INSPIRATION 10 SECONDS EXPIRATION 10 SECONDS 1 MOVEMENT		INSPIRATION 5 SECONDS EXPIRATION 5 SECONDS 2 MOVEMENTS		INSPIRATION 3½ SECONDS EXPIRATION 3½ SECONDS 3 MOVEMENTS	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.78–5.86	155	3.66–5.78	158	3.43–5.66	165	3.32–5.68	171
3.27–5.00	153	3.40–5.20	153	3.66–5.82	159	3.47–5.79	167
3.56–5.33	149	3.76–6.14	165	3.67–6.13	167	3.37–5.70	169
3.56–5.52	155	3.54–5.35	154	3.54–5.49	155	3.25–5.59	172
3.62–5.29	146	3.41–5.35	157	3.47–5.79	167	3.33–5.73	172
3.65–5.55	152	3.68–5.59	152				
Average = 152		Average = 157		Average = 163		Average = 170	

occurring at each act of inspiration. The results in tables 3 and 4 show that this is indeed the case.

Boothby and Peabody (9) in connection with the determination of the alveolar CO_2 by the Plesch-Higgins method found that the rate and depth of respiration while rebreathing do not play a very important rôle, in that the CO_2 tension after five moderately deep or five

very deep respirations in 25 seconds was about the same as that obtained after 16 or 17 shallow respirations in the same time. The CO_2 tension, however, was considerably greater than when the breathing was "natural." It may be stated here that the CO_2 tensions which these authors give, (with the exception of the value obtained with five "natural" respirations in 25 seconds) are all quite high, and probably represent the CO_2 tension of the "venous pulmonary air" rather than that of the "arterial pulmonary air," as Henderson and Prince have suggested the determinations by the Plesch-Higgins method are likely to do.

Finally in order to ascertain whether variations in intra-pulmonic pressure have any effect, directly or indirectly, upon the rate of CO_2 exchange between the blood and the air in the lungs, a series of determinations of the CO_2 tension in the air was made after the breath had been held under positive and negative pressures. The results are shown in table 5. The CO_2 tension is the same after the breath has been held for 20 seconds under positive pressures of 10, 20 or 30 mm. Hg. as when it is held under atmospheric pressure. But when the breath is held under negative pressure the CO_2 tension shows a considerable increase.

These results are similar to those obtained by Wardlaw. Up to negative pressures of 10 mm. Hg. he found that the increase in CO_2 tension was proportional to the pressure. Above this pressure the increase in CO_2 was found to be practically constant for all pressures. Wardlaw obtained practically the same increase in the rate of the gaseous exchange when the breath was held under pressures greater than -10 mm. Hg. as when the air was rebreathed. This fact he cites as additional evidence that the increased respiratory exchange caused by the movements of breathing is not brought about by a quickening of the circulation. Now if the percentage of increase of CO_2 when the air is rebreathed three times in 20 seconds is noted (see tables 3 and 4), it is seen that it is only a little lower than when the breath is held under negative pressures for the same length of time (see table 5).

The bearing of these and Wardlaw's results of a similar kind on indicating that the increased gaseous exchange is not due to a quickening of the circulation, as Wardlaw claims, is not clear to me. The fact, which Wardlaw apparently neglects to consider, is not that the increased circulation necessarily increases the rate of gaseous exchange but that the decreased rate of circulation, or the diminution of the blood volume, when the breath is held, decreases the rate of gaseous

exchange. During rebreathing the pressure conditions approach more nearly those holding under normal breathing where at each inspiration the circulation rate is hastened, and at each expiration, retarded.

It may be that when the negative intrapulmonic pressure is increased by holding the breath under a negative pressure, the retarding effect on the gaseous exchange due to the mechanical obstruction to the circulation is offset by the greater than normal negative intrapulmonic pressure.

TABLE 5

Comparison of the rise of CO_2 tension when the breath is held for 20 seconds under atmospheric pressure, and under various positive and negative pressures

ATMOSPHERIC		+ 10 MM. Hg		+ 20 MM. Hg		+ 30 MM. Hg	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.66–5.72	156	3.53–5.11	144	3.66–5.44	149	3.69–5.41	147
3.82–5.75	151	3.70–5.70	154	3.74–5.69	153	3.81–5.52	145
3.74–5.69	152	3.89–5.83	150	3.71–5.62	152	3.82–5.81	152
3.81–5.70	150	3.76–5.85	156	3.73–5.65	151	3.62–5.59	162
3.58–5.68	159	3.63–5.84	161	3.69–5.58	151	3.55–5.75	162
Average = 154		Average = 153		Average = 151		Average = 152	
ATMOSPHERIC		− 10 MM. Hg		− 20 MM. Hg		− 30 MM. Hg	
Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase	Increase in per cent of CO_2	Per cent of increase
3.71–5.75	155	3.70–6.17	167	3.75–6.24	167	3.51–6.02	172
3.72–5.74	155	3.55–6.12	172	3.75–6.21	166	3.46–6.19	176
3.36–5.24	156	3.69–6.39	173	3.43–6.21	181	3.56–6.35	178
3.59–5.56	155	3.77–6.43	171	3.85–6.40	171	3.33–5.99	180
3.78–5.71	151	3.61–6.04	167	3.66–6.42	175	3.73–6.51	175
Average = 154		Average = 170		Average = 172		Average = 176	

It might be thought that the higher tension of CO_2 after rebreathing air as compared with the tension attained after holding the breath was due to the influence of oxygenation. But, as Wardlaw's results show, the oxygen tension falls at a more rapid rate than that at which the CO_2 rises, and falls more rapidly when the air is rebreathed than when the breath is held. Furthermore when the breath is held long enough the CO_2 tension attains a certain fixed value while the oxygen tension continues to fall during the whole period for which the breath can be held or the air rebreathed.

Hill and Flack found too when the breath was held that the fall in the percentage of oxygen is greater than the rise of CO_2. Also that when oxygen is held instead of air, the tension of CO_2 at the breaking point is raised. Furthermore that when the air is rebreathed, the CO_2 goes higher and the oxygen tension lower than when the breath is held, and again that when oxygen is rebreathed a much higher tension of CO_2 is reached.

Now the higher tension of CO_2 reached when oxygen is held or rebreathed is undoubtedly due to the influence of oxygenation (see Christiansen, Douglas and Haldane), but it does not follow that the higher percentage of CO_2 attained after rebreathing than after holding the breath is due to the same cause, because the oxygen tension falls more rapidly in the former case and therefore the higher percentage of CO_2 could not be due to the forcing of CO_2 out by the higher tension of oxygen, but rather the de-oxygenation of the blood in the tissues would help the absorption of CO_2 and diminish its rise of pressure.

It seems to me that the results of Hill and Flack indicate clearly that holding the breath produces some mechanical obstruction to the circulation by the cessation of the respiratory pump. In rebreathing, the blood is circulated more freely and CO_2 is therefore more quickly, and in greater amount, removed from the tissues than when the breath is held.

DETERMINATION OF THE CO_2 TENSION OF THE VENOUS PULMONARY AIR

It is evident from the experiments described above, as well as from the work of Christiansen, Douglas and Haldane and that of Wardlaw, that neither holding the breath nor rebreathing air from and into a closed bag, yields CO_2 tensions which can be considered as representing the CO_2 tensions of the venous pulmonary air. They are therefore of no practical value except in so far as they can be compared with and controlled by other methods.

The next point to determine was whether the method of Henderson and Prince gives results which represent the CO_2 tension of the venous pulmonary air.

Their method contains two variations, viz., *a*, holding the breath for a certain number of seconds at each successive intermittent rebreathing, and *b*, inspiring it for 5 seconds and expiring it for 5 seconds every time that it is intermittently rebreathed. These two methods have been compared in some detail, examples of the results being shown in

tables 6 and 7. In table 6 CO_2 tensions obtained after intermittent holding of the breath for 5, 10 and 15 seconds are given. The CO_2 in the air in my lungs seems to be in equilibrium with the blood when its tension reaches between 6.10 and 6.30 per cent, (average 6.24 per cent,

TABLE 6

The effect of intermittent rebreathing—holding the breath—on the rise of CO_2 tension

NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2					
	Held for 5 seconds	Held for 5 seconds	Held for 10 seconds	Held for 10 seconds	Held for 15 seconds	Held for 15 seconds
0	3.60	3.35	3.40	3.61	3.11	3.68
1	4.31	4.22	4.76	4.96	4.63	5.65
2	4.95	4.90	6.14	5.95	6.05	6.29
3	5.21	5.44	6.32	6.19	6.14	6.33
4	5.44	5.71	6.36	6.11	6.34	6.33
5	5.65	5.96	6.39	6.13	6.28	6.30
6	5.93	6.07	6.26			
7	6.11	6.09	6.26			
8	6.13	6.11	6.21			
9	6.10	6.10				
10	6.12					

TABLE 7

The effect of intermittent rebreathing—inspiring for 5 seconds, and expiring for 5 seconds—on the rise of CO_2 tension

NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2				
	1 respiration	1 respiration	2 respirations	2 respirations	3 respirations
0	3.41	3.57	3.31	3.19	3.61
1	4.77	4.92	5.06	5.08	6.35
2	6.01	5.90	6.10	6.03	6.79
3	6.13	5.94	6.47	6.50	6.91
4	6.20	6.11	6.78	6.58	7.12
5	6.33	6.09	6.63	6.56	7.12
6	6.31	6.08	6.64	6.63	7.10
7	6.28	6.10	6.70		
8			6.61		
9			6.63		

or 44.9 mm. Hg.), which occurs after the 5th, 6th or 7th intermittent rebreathing. The longer the air is held at each intermittent rebreathing, the more rapidly does the CO_2 tension increase (see held for 5, 10 and 15 seconds and fig. 2), the final constant value is however the same.

Every time that the air in the bag was rebreathed it must have been diluted to a small extent by the air in the dead space and to a slighter extent by the residual air, at least after the percentage of CO_2 in the rebreathed air had become greater than that of the arterial pulmonary air. Determination of the CO_2 tensions of the alveolar air, that is the last portions of an expiration after the air had been intermittently rebreathed until the CO_2 tension had become constant, by the method of Henderson and Morriss (10) gave, however, practically the same values for the CO_2 tension as analysis of the "mixed air" did.

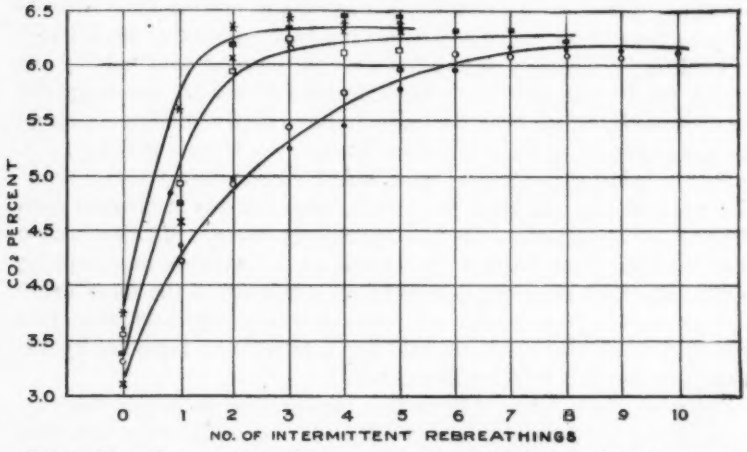

Fig. 2. The effect of intermittent rebreathing—holding the breath—on the rise of CO_2 tension; ● ○ when the breath is held for 5 seconds, ■ □ when the breath is held for 10 seconds, × ★ when the breath is held for 15 seconds.

Christiansen, Douglas and Haldane point out the marked influence that oxygenation has upon the dissociation curve of CO_2. In the lungs the amount of CO_2 given off is increased by about 50 per cent, while in the tissues the oxygen reduces by about 40 per cent the amount of CO_2 given off to the venous blood. The difference therefore between the CO_2 tensions of arterial and venous blood is reduced by about 40 per cent, and these authors had to take this into consideration in the determination of the venous CO_2 by their method. Boothby and Sandiford (3) also took care that there was sufficient oxygen in the mixtures which they held in the lungs to insure complete saturation of the Hb. In the method proposed by Henderson and Prince for the

determination of the venous CO_2 tension the influence of oxygenation does not come into consideration.

The CO_2 tension of my arterial pulmonary air is 5.53 per cent. There is therefore a difference of 0.71 per cent (6.24–5.53), or 5.1 mm. Hg. (44.9–39.8), between the CO_2 tension of my arterial and of my venous blood, a figure quite close to the average differences found by Christiansen, Douglas and Haldane.

As mentioned above, Henderson and Prince suggested, as a variation on holding the breath every time that it was intermittently rebreathed, that it be inspired for 5 seconds and expired for 5 seconds "one or more times." This variation has been repeated to see whether it would give the same values for the CO_2 tensions that holding the breath for 10 seconds does. When, instead of holding the lungs full for 10 seconds, the air from the bag is inspired for 5 seconds and expired for 5 seconds, once, the same value for the CO_2 tension is found, (Cf. table 6, "held for 10 seconds" and table 7 "1 respiratory movement;" also see table 3). Holding the breath, after a sharp inspiration from the bag, for 5 seconds results in a percentage increase of CO_2 less than that resulting from holding the breath for 10 seconds, but inspiring continually for 5 seconds followed by an expiration of the same length of time results in a percentage increase which is greater than that obtained when the breath is held for 5 seconds and practically the same as when it is held for 10 seconds.

But when the number of times that the air is rebreathed in this way, by inspiring for 5 seconds and expiring for 5 seconds, every time that the air is taken into the lungs from the bag, is more than one, it is found that the rate of CO_2 increase is greater, as well as the final constant value attained (see table 7 and fig. 3). This is, of course, just what might have been expected from the results that have been described above on rebreathing as compared with holding the breath, as well as from the fact that the total length of time that the air is rebreathed is considerably increased. It is interesting to note that a constant CO_2 tension, higher as it is, is however reached.

The statement by Henderson and Prince regarding this variation is ambiguous. They probably did not mean that the air should be inspired and expired in this way more than once at each successive intermittent rebreathing.

The application of the CO_2 tension of the venous pulmonary air, as well as that of the arterial pulmonary air, to the Plesch-Higgins method is of clinical importance. Knowing the venous CO_2 tension, this

application can be made with a degree of certainty. As has been shown above the length of time that the breath is held in the lungs, as well as the number of respiratory movements in unit time, have an influence on the CO_2 tension of the expired air.

Reference was made above to the results of Boothby and Peabody (9) on the relation of the CO_2 tension to the rate and depth of respira-

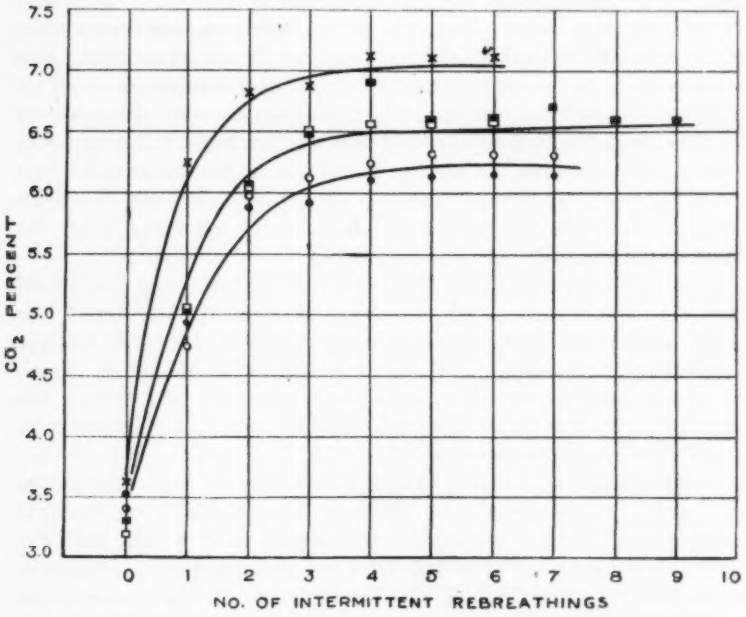

Fig. 3. The effect of intermittent rebreathing—inspiring for 5 seconds and expiring for 5 seconds—on the rise of CO_2 tension; ● ○ when one respiratory movement is made, ■ □ when two respiratory movements are made, × when three respiratory movements are made.

tion, and the suggestion made that the values of the CO_2 tensions which they obtained by the Plesch-Higgins method approximate the venous rather than the arterial, as compared with the tensions obtained by the Haldane method. Also reasons for doubting the validity of Wardlaw's results concerning the frequency of respiratory movement have also been given above, as well as evidence showing that increasing the number of respirations in a certain period of time—keeping

the amplitude as constant as possible—raises the CO_2 tension of the expired air. Henderson and Prince (1) expressed the opinion that the Plesch-Higgins method of determining the alveolar CO_2 gives results which are too high. Further, that by this method of rebreathing the portion of the curve of CO_2 percentage where it begins to become level represents the CO_2 tension of the tissues. Table 2 and figure 1 of the present paper show that the beginning of the nearly horizontal part of the curve, or the approximate constancy of CO_2 comes after the 9th rebreathing, with an average CO_2 tension of 7.15 per cent. The CO_2 tension of my arterial pulmonary air is about 5.53 per cent. This tension is reached by the method of continuous rebreathing, somewhere between the 2nd and 3rd rebreathing (see table 2, 3rd and 4th, if the air expired into the bag be considered as having already been rebreathed once), and the venous CO_2 tension (6.24 per cent) is usually passed between the 4th and 5th rebreathing (or 5th and 6th for the above stated reason).

One of the regular exercises of the class in physiology in this school consists in the determination of the gas tensions of the arterial pulmonary air by the Haldane-Priestley, Henderson and Morriss and the Plesch-Higgins methods. The results obtained by Mr. B. E. Read, one of the members of the present year's class, are as follows. His arterial CO_2 tension by the Haldane-Priestley method is 5.72 per cent, by the Henderson and Morriss, 5.77. The CO_2 tensions after continuous rebreathing once every 5 seconds are given in table 8. The values given are the average of four or more determinations on separate rebreathings. The air rebreathed was expired into a bag, and was always somewhat more than the tidal volume in amount. After the air has been rebreathed twice, the CO_2 tension reaches that determined by the Haldane and Henderson methods. (If the air expired into the bag be considered as already having been rebreathed once, then the arterial CO_2 tension is reached after the 3rd continuous rebreathing).

In addition B. E. R. has determined the CO_2 tension of his venous pulmonary air by the method of intermittent rebreathing. This was found to be about 6.34 per cent. As seen from the values for continuous rebreathing given in table 8, the venous CO_2 is reached after the 3rd continuous rebreathing, (or the 4th, for the above given reason).

The following method of obtaining the CO_2 tension of the venous pulmonary air and which is applicable to clinical patients, is suggested. A bag sufficiently large to hold enough air to fill the lungs when inhaled is filled with fresh air, by water displacement or by a pump. This air

is taken into the lungs after they have been emptied as far as possible, and then immediately exhaled into the bag and a sample analyzed for CO_2, (found, in my case, to vary between 3.25 and 4.0 per cent). Or the subject can exhale lightly, with pauses, two or three times into the bag, inspiring from the outside air, and the air in the bag then be analyzed for CO_2. This air should now be intermittently rebreathed,—with sufficiently long pauses between successive rebreathings to allow the circulation and respiration to become normal,—according to the Henderson and Prince method, of inspiring for 5 seconds and expiring for 5 seconds, until on subsequent rebreathings and analyses a constant CO_2 percentage is reached. Since the same results have been shown to be obtained when the breath is held for 10 seconds as when the air

TABLE 8

The rise of CO_2 tension after continuous and intermittent rebreathing

NUMBER OF REBREATHINGS	PERCENTAGE OF CO_2		
	B. E. R.		E. F. A.
	Continuous rebreathing	Intermittent rebreathing	Intermittent rebreathing
1	4.62	4.41	4.34
2	5.73	5.27	5.69
3	6.21	5.93	6.39
4	6.82	6.31	6.43
5	6.91	6.34	6.60
6			6.56

is inspired for 5 seconds and expired for 5 seconds once for each intermittent rebreathing (see tables 6 and 7), the latter method, owing to the greater ease with which it can be carried out by sick patients is preferable for them.

Furthermore by this same procedure the CO_2 tension of the arterial pulmonary air can be approximately determined. As tables 6 and 7 show, when the air after inspiring it from the bag, is held for 10 seconds and then expired into the bag; or when it is inspired for 5 seconds and expired for 5 seconds, at each intermittent rebreathing, a CO_2 tension approximately equal to that of the arterial pulmonary air is passed between the 1st and 2nd intermittent rebreathing. By adding the tensions found after the 1st and 2nd rebreathing, and halving them, the approximate CO_2 tension of the arterial pulmonary air can be obtained. The average obtained when the breath is held for 10 sec-

onds is 5.46 per cent; when an inspiration and expiration, each lasting 5 seconds, is made at each intermittent rebreathing, an average of 5.41.

An even more approximate determination of the CO_2 tension of the arterial pulmonary air can be obtained by taking the average of the CO_2 tensions of the first two rebreathings, and the average of the first three, and then taking the average of these. When the breath is held for 10 seconds at each intermittent rebreathing (see table 6) a value of 5.59 is thus obtained, and when the air is inspired for 5 seconds and expired for 5 seconds once at each intermittent rebreathing (see table 7) a value of 5.54 per cent. By applying this calculation to the results obtained on two other subjects (see table 8) a percentage value of 5.72 is obtained for B. E. R., which is identical with the CO_2 tension obtained by the Haldane method; and of 5.24 for E. F. A., whose arterial pulmonary air by the Haldane method has a percentage value of 5.36.

SUMMARY

1. The rise of CO_2 tension in the lungs when the breath is held is compared with that when the air is rebreathed.

2. The CO_2 in both increases at a gradually decreasing rate until it reaches an apparent maximum. The rate of increase and the final value attained are higher when the air is rebreathed than when it is simply held in the lungs. The time that the air can be rebreathed is also longer than that for which it can be held, although the CO_2 is higher.

3. The method suggested by Henderson and Prince for the determination of the venous CO_2 tension has been carefully gone over and the two variations suggested by them of intermittent rebreathing compared.

4. The Plesch-Higgins method of determining the CO_2 tension of the alveolar air is shown to give results which are too high when the air is rebreathed more than two to three times, and approximately equal to the venous CO_2 tensions when four to five rebreathings are made.

5. A method for determining the venous CO_2 tension (slightly modified from that of Henderson and Prince) and applicable to clinical patients, is suggested, as well as for the calculation of the approximate arterial CO_2 tension.

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LOCATION OF THE ADRENALIN VASODILATOR MECHANISMS*

FRANK A. HARTMAN, LESLIE G. KILBORN AND LOIS FRASER

From the Laboratory of Physiology of the University of Toronto

Received for publication March 26, 1918

It has been demonstrated (1) that adrenalin vasodilatation in the limb can be produced by stimulation of other than peripheral structures. It has also been shown that intestinal vasodilatation from adrenalin involves either the collateral ganglia or the central nervous system. These facts were established, *a*, by cutting the nerves to the limb in the one case and by destroying the ganglia in the other; *b*, by perfusion experiments in which the organ is cut off from the body circulation and the nerves left intact, adrenalin being injected into the jugular vein.

Previous to the present research we had found evidence which indicated a difference between the type of mechanism causing vasodilatation in the limb, as an example of skeletal muscle, and that producing like effect in the intestine: viz., 1, small doses of adrenalin produce constriction in the intestine and dilatation in the limb while larger doses produce the reverse effect, i.e., dilatation in the intestine and constriction in the limb (1, p. 366); 2, greatly increasing the dose above that causing intestinal dilatation does not produce predominant constriction in the intestine; 3, the intestinal vasodilator mechanism develops later than the corresponding mechanism for the limb (2).

In the present research we have attempted to determine the location of these mechanisms.

The procedure followed was to destroy different portions of the brain and spinal cord, to remove the sympathetic ganglia or to destroy the dorsal root ganglia of the nerves from the organ investigated and then to ascertain the activity of the vasodilator mechanisms according to methods described in previous investigations (1).

Removal of cerebrum and cerebellum. A cat (2.8 kgm.) responded to 0.2 cc., 1: 100,000 adrenalin with a fall in blood pressure of 19.3 per cent (150 mm. to 121 mm.). Nine minutes after removal of the cere-

brum a similar dose of adrenalin produced the same percentage drop in blood pressure (114 mm. to 92 mm.) This indicates that the adrenalin vasodilator mechanisms are not in the cerebrum, at least those which control the vessels of skeletal muscle and are called into play by small doses of adrenalin. In order to confirm our conclusion in regard to the position of these, we studied the volume changes in the hind limb of one cat and one dog. Dilatation of the limb of the cat from adrenalin occurred after decerebration as well as before. A similar result was obtained in the dog after removal of both cerebrum and cerebellum.

Blood pressure changes, however, do not indicate the action of the intestine, therefore in order to discover whether the intestinal mechanism was present in the cerebrum the volumetric method was necessary. In both animals the intestinal dilatation thresholds were determined before decerebration. The cerebrum was destroyed in the cat, then the same dose of adrenalin was injected as before with like result, showing that the intestinal mechanism was not in the cerebrum. The dog had both the cerebrum and the cerebellum removed without interfering with the intestinal dilatation.

We are justified, therefore, in concluding that neither type of the adrenalin vasodilator mechanisms is present in the cerebrum or cerebellum.

Destruction of the medulla. Our next step was to destroy the medulla. This was done in those animals which had served for the cerebral experiments, and in some others in which the brain was pithed in one operation. (In either case the ether was immediately discontinued.) Destruction of the medulla always produced a reversal in the blood pressure response to adrenalin, in none was there a fall in blood pressure (four dogs and eight cats). It appeared, therefore, that the dilator mechanisms might be situated in this region of the central nervous system. A typical example is as follows:—before pithing the brain, 0.2 cc., 1: 100,000 adrenalin produced a 14 per cent fall in blood pressure in a cat (166 mm. to 152 mm.); twice the amount produced a 30 per cent fall. After pithing, the same doses of adrenalin produced 10 per cent and 32 per cent rises in blood pressure, respectively (from 78 mm.). One of us has shown (3), however, that a decrease in the blood pressure (e.g., by hemorrhage) is enough in itself to produce a similar result. The reversal in the reaction in this case then was not necessarily due to destruction of the dilator mechanisms. We sought an answer to this question by a study of the volume changes of the

organs. The dilatation of the limb muscles, recorded with the plethysmograph, in two dogs and four cats followed the curve of the rise in blood pressure and seemed to be passive. Intestinal volume changes were recorded in seven subjects (two dogs and five cats). In all but one the dilatation was active, independent of the rise in pressure.

In order to exclude all possibility of passive dilatations, the hind limb of a cat was perfused with warm oxygenated Ringer's solution through the common iliac artery. The abdominal aorta was clamped high up to prevent anastomoses. The vena cava was tied and an outlet made in the common iliac vein (1, p. 360). The response of the perfused limb to various doses of adrenalin injected into the general circulation was noted, after which the brain was pithed and the injections repeated. The resulting dilatation was in every case as marked as before. Similarly the perfused hind limb of a dog responded by dilatation as well after destruction of the brain as before. A perfused intestinal loop of a brainless dog dilated when adrenalin was injected into the general circulation.

Cervical cord. Having failed to locate the vasodilator mechanisms in the medulla or higher, their presence in the cord of the cervical region seemed very doubtful. The upper part of the central nervous system down to the thoracic cord was destroyed by pithing in a dog and a cat. The limb mechanism in the dog was active, as determined by the plethysmograph after pithing. The intestinal reaction of the dog was not studied but in the cat it was still present. It must be concluded that both mechanisms are below the cervical cord.

Thoracic cord. After destruction of the central nervous system as far down as the mid-thoracic region the adrenalin vasodilator mechanism for the intestine still worked in every case (four cats and one dog). It appeared, therefore, that the intestinal mechanism must be located below this region.

The cord was next pithed to the lumbar region in three cats and one dog. Adrenalin still caused intestinal dilatation in all cases, though in the dog the dilatation was not as marked as before. Therefore the mechanism for adrenalin vasodilatation in the intestine appeared to lie outside of the brain and spinal cord (fig. 1).

The adrenalin vasodilator mechanism for the hind limb is not located in the thoracic cord. This was proved in both normal and perfused limbs by destruction of the brain and cord. In the animals with the normal limb (two cats and one dog) one seemed to respond by passive dilatation while the others were active. To avoid passive effects the

hind limb was perfused after destruction of the brain and cord to the lumbar level, (one cat and one dog). Injection of adrenalin into the jugular vein caused dilatation in the perfused limb in each case.

Lumbar cord. The brain and spinal cord were completely pithed in two cats. Adrenalin produced dilatation in the hind limb in both. However, this seemed to be passive. The lumbar and sacral cord only were destroyed in a third cat without preventing dilatation of the hind limb from adrenalin. Where the limb effects appear to be passive a distinction can be shown by using a denervated limb. The

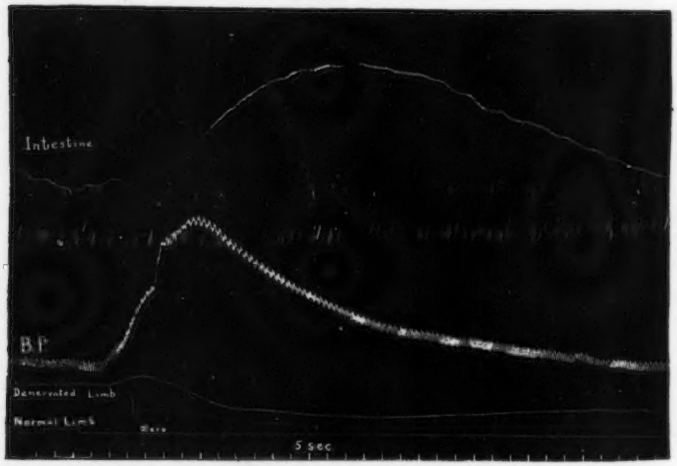

Fig. 1. The reaction of normal limb, denervated limb and intestine in a cat (weight 3 kgm.) to 3 cc., 1: 100,000 adrenalin after destruction of the brain and spinal cord. Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

latter gives earlier and more marked constriction than does the normal limb (fig. 1). We again found it necessary to resort to perfusion experiments. In addition the pithing was done with a stiff brush to insure complete destruction of the cord.

The lumbar and sacral regions of the cord were destroyed in two dogs. One hind limb was perfused and the abdominal aorta clamped. In both experiments good dilatations were obtained from injecting adrenalin into the jugular vein.

The sacral, lumbar and lower half of the thoracic cord were destroyed

in two cats. The perfused hind limb in each case dilated when adrenalin was injected into the jugular vein (fig. 2). The dose of adrenalin necessary to do this was larger than is the case in a normal limb, perhaps because of the faulty circulation in the lumbar region caused by clamping the aorta above the bifurcation. We do not find the same difference after the operation in dogs as in cats, owing no doubt partly to the great number of anastomoses in a larger animal.

These results were confirmed by experiments in which the connection between the central nervous system and the limb under observation was severed, after which no reduction was found in the dilatation caused by adrenalin. We chose dogs for this operation. The spinal

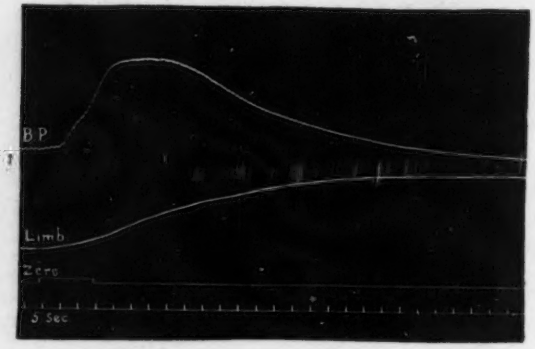

Fig. 2. Dilatation of a perfused hind limb in a cat (weight 2.5 kgm.) to 1 cc., 1: 10,000 adrenalin injected into the jugular vein after complete destruction of the spinal cord downward from the eighth thoracic level. Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

nerve roots were exposed on one side by removing the laminae and part of the transverse processes, but not the spines. Bleeding from the sinuses was stopped by hot saline packs from time to time. In the first experiment both dorsal and ventral roots of the sacral and lumbar regions were cut close to the cord on one side. The limb of that side was placed in a plethysmograph and perfused. The aorta and vena cava were tied. Injection of 0.5 cc., 1: 20,000 adrenalin into the jugular vein caused a pronounced dilatation of the perfused limb, in spite of the low blood pressure which had resulted from hemorrhage, and succeeding doses of the same strength had a similar effect. 2 cc., 1: 20,000 adrenalin caused dilatation which persisted for some time

(fig. 3). A second dog was studied in a similar manner with the same result.

We therefore came to the conclusion that the vasodilator mechanism for the hind limb could not be situated within the central nervous system but must lie either in the sympathetic or in the dorsal root ganglia or, perhaps, in both.

Location of the mechanism for skeletal muscle. Study of the sympathetic ganglia was next made. The right hind limb of a dog (26.0 kgm.) was placed in a plethysmograph. The last five lumbar and the

Fig. 3. Reaction of the perfused hind limb of a dog (weight 12.6 kgm.) to 2 cc., 1: 20,000 adrenalin injected into the jugular vein after cutting both dorsal and ventral roots central to the dorsal root ganglia. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

first sacral sympathetic ganglia were destroyed on the right side. The limb was next completely shut off from the circulation and perfused with warm oxygenated Ringer's solution. Injection of 1.5 cc. of 1: 20,000 adrenalin into the jugular vein caused slight dilatation of the perfused limb, while after twice the dose the dilatation was marked. A second animal was studied after destruction of the last five lumbar and the first two sacral sympathetic ganglia. The dilatation from

adrenalin was very great (fig. 4). In a third dog both sympathetic chains were completely destroyed on both sides from the third lumbar ganglion downward. Adrenalin, injected into the general circulation, caused the perfused limb to dilate as in the other experiments. The animals were always examined at the completion of the experiments to ascertain the limit of gangliar destruction.

These experiments made it appear that the limb mechanism was not in the sympathetic ganglia but in those of the dorsal roots. To determine this we approached the question in another way. The

Fig. 4. Dilatation in the perfused hind limb of a dog (weight 21.6 kgm.) from the injection of 4 cc., 1: 20,000 adrenalin into the jugular vein, after removing the last five lumbar and the first two sacral sympathetic ganglia on the same side. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

dorsal and ventral roots of all lumbar and sacral nerves were cut central to the dorsal root ganglia on the right side. The right hind limb, after being placed in a plethysmograph, was completely cut off from the general circulation and immediately perfused. Adrenalin injected into the jugular vein caused dilatation of the perfused limb. The next step was removal of all dorsal root ganglia supplying the perfused limb. Following this operation injection of adrenalin into the jugular vein produced an effect on the perfused limb similar to that occurring before removal of the ganglia (see fig. 5). This was repeated in three dogs with the same result each time except that in one animal, in which

the blood pressure became quite low after removal of the dorsal root ganglia, somewhat larger doses of adrenalin were required to produce dilatation as large as before; this may perhaps be due to the poor circulation to the sympathetic ganglia because of the clamp on the abdomi-

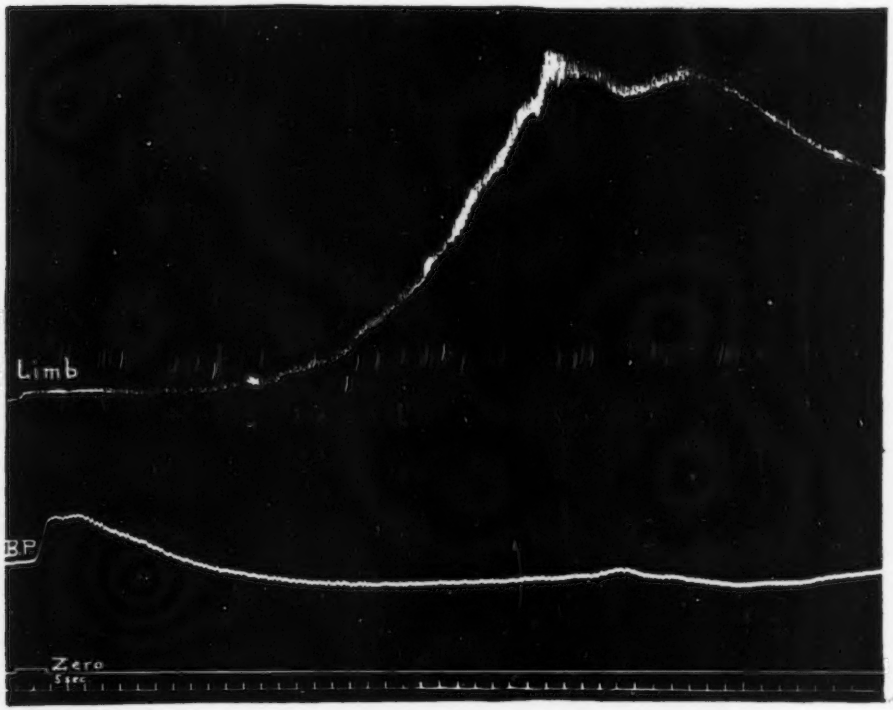

Fig. 5. Dilatation of a perfused hind limb (dog 9.0 kgm.) due to the injection of 2 cc., 1: 20,000 adrenalin into the general circulation. All dorsal root ganglia had been removed after cutting the dorsal and ventral nerve roots in the whole lumbar and sacral region on the side of the perfused limb. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

nal aorta and the general low blood pressure. We have indeed evidence of interference with the circulation of blood to the sympathetic ganglia in the delayed dilatation of the limb, i.e., in some animals the limb began to dilate long after the beginning of the change in blood pressure.

Having shown that the adrenalin vasodilator mechanism for the hind limb is in part at least to be found in the sympathetic ganglia, we next proved that the dorsal root ganglia were also in part responsible for the dilatation when adrenalin was injected. In the experiments where the sympathetic chains to the perfused limb had been completely destroyed, the remaining adrenalin vasodilator mechanisms must have been either in the dorsal root ganglia or in the spinal cord. Since from the results of our experiments on destruction of the central nervous system we were convinced that it did not contain the seat of the reaction, we wished to have definite proof that this was to be found in the dorsal root ganglia. This proof we got by destroying both abdominal and sacral sympathetic chains and cutting both dorsal and ventral roots central to the dorsal root ganglia in the whole lumbar and sacral region on the side from which the perfused limb received its supply.[1] Four dogs were studied after the above operation. In each case adrenalin injected into the general circulation caused dilatation of the perfused limb (see fig. 6). In two of the animals we then removed the dorsal root ganglia, whereupon adrenalin when injected into the general circulation failed to produce any effect upon the perfused limb.

We were able to confirm the location of the adrenalin vasodilator mechanisms for the hind limb in both sympathetic and dorsal root ganglia by the direct application of adrenalin to them.

The influence of adrenalin upon the sympathetic ganglia of the lumbar region was studied in three cats. The last two lumbar ganglia were exposed by careful dissection. A small funnel was clamped in such a position that the outlet was over one of the ganglia so that small amounts of adrenalin, poured down the funnel, bathed it. Be-

[1] We were unable to obtain satisfactory results in cats in most cases after exposure of the dorsal root ganglia, as the following experiments show. We cut dorsal nerve roots central to the ganglia in two cats on one side. In one all roots were cut from the sacral to the mid-thoracic, in the other all to the thoracic level were cut. Then the hind limb on the corresponding side was placed in a plethysmograph and perfused. In neither animal could dilatation of the perfused limb be obtained from injection of adrenalin into the general circulation. In a third cat the lumbar and sacral cord was merely exposed, after which a hind limb was placed in a plethysmograph and perfused. Even in this case no dilatation could be obtained in the perfused limb when adrenalin was injected into the general circulation, although the blood pressure was about normal (142 mm.) In the two preceding cases the blood pressure was so low that it was thought possibly a factor, (18 mm.)

tween applications the adrenalin was washed out with isotonic salt solution and taken up with absorbent cotton. The volume of the limb was recorded by means of a plethysmograph. In the first animal a 1: 100,000 solution of adrenalin produced a good dilatation of the limb with hardly any blood pressure change. A 1:10,000 solution produced marked constriction of the limb together with a steady rise in blood pressure. We consider that this constriction was not necessarily a local effect at the ganglion because of the pronounced blood pressure change which accompanied it. The second animal gave dilatation of the limb upon the first application of 1: 100,000 adrenalin, but later applications of the same concentration were without effect.

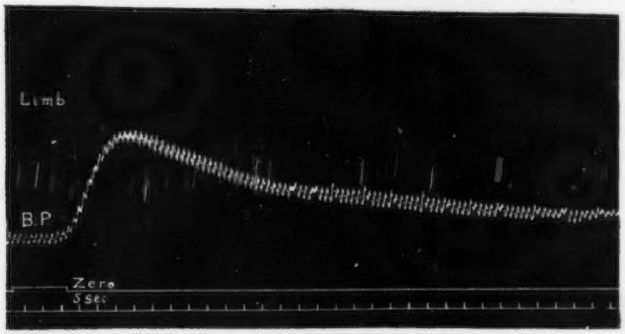

Fig. 6. Dilatation of a perfused hind limb (dog 14. kgm.) from the injection of 5.5 cc., 1: 10,000 adrenalin into the jugular vein. All sympathetic ganglia on both sides in the lumbar and sacral regions had been destroyed and all dorsal and ventral nerve roots central to the dorsal root ganglia had been cut below the thoracic level on the side of the perfused limb. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

In the third animal a 1: 100,000 solution had no effect. A 1: 10,000 solution caused a slight dilatation of the limb and a fall in blood pressure from 105 mm. to 102 mm. A 1: 5000 solution caused a more pronounced diltation of the limb and a fall in blood pressure of 25 mm. (fig. 7). A 1: 1000 solution caused marked dilatation of the limb followed later by constriction. The blood pressure change in this case was a pure fall of 21 mm.

It was more difficult to produce dilatation of the hind limb by the application of adrenalin to the dorsal root ganglia. Three dogs were studied. The lower lumbar ganglia were used, the sheaths covering

them were slit and sometimes the ganglia themselves in order to permit better access of the adrenalin. In all cases both dorsal and ventral roots were cut central to the ganglia to prevent any possible effect from the cord. The solution of adrenalin was washed away with isotonic salt solution between each application. In the first experiment, a solution of 1: 10,000 adrenalin produced a doubtful dilatation. A second dose of the same concentration produced no effect nor did stronger concentration cause any change. In a second experiment we met with greater success. Although 1: 10,000 solutions produced no effect, those of 1: 1000 caused dilatation in the limb and repeated applications of solutions of this concentration always produced the same effect. In a third case dilatation of the hind limb

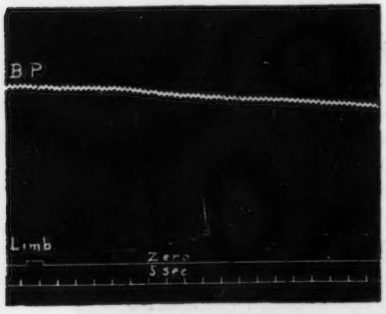

Fig. 7. Dilatation of a hind limb due to direct application of 1: 5000 adrenalin to the sixth and seventh lumbar sympathetic ganglia. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

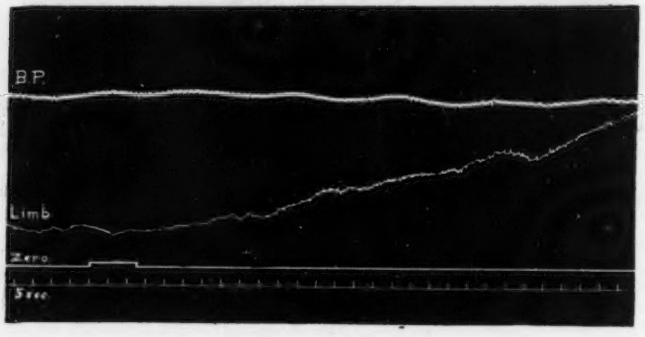

Fig. 8. Dilatation of a hind limb due to direct application of 1: 1000 adrenalin to one of the lower lumbar dorsal root ganglia. Dog, 16 kgm. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

was obtained time after time upon application of 1: 1000 adrenalin to the lower dorsal root ganglia (see fig. 8).

Location of the intestinal mechanism. We have previously shown (4) that the intestinal vasodilator mechanism does not function after destruction of the semilunar and superior mesenteric ganglia. At that time we stated that cutting of the splanchnic nerves produced the

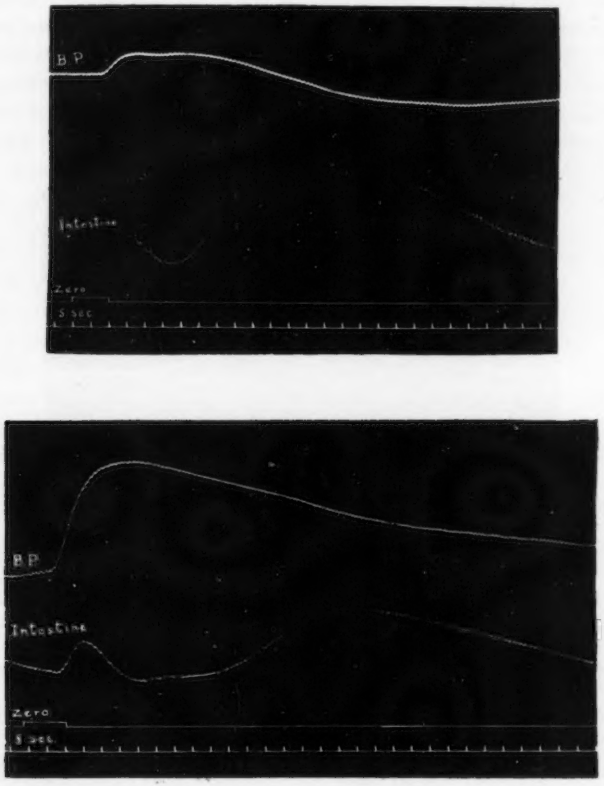

Fig. 9. Dilatation of intestine from adrenalin persists after cutting all splanchnic fibers, though it may be reduced. Upper record is the response to 0.5 cc., 1: 10,000 adrenalin before cutting the splanchnic fibers. Lower record is the response to 1 cc., 1: 10,000 adrenalin after cutting the splanchnic fibers in the same animal. (Cat weight 2.5 kgm.) Base of bellows 26 mm. x 13 mm. (Reduced one-half.)

same result, judging from the result of the one experiment of this kind (the other experiments of the series were gangliar destruction).

It now appears from further experiments that cutting the splanchnics does not necessarily do away with the dilatation. We divided the splanchnic nerves in five cats and took records of the reaction of the intestine to adrenalin. In one the reaction was constriction only; the remaining four gave dilatation as before except that in one animal it was less marked (fig. 9) Destruction of the semilunar and superior mesenteric ganglia in one of these greatly reduced the dilatation from adrenalin but did not quite abolish it. Since, however, section of all nerves in the stalk of this loop did not prevent a small amount of dilatation, we concluded that some small part of the previous dilatation

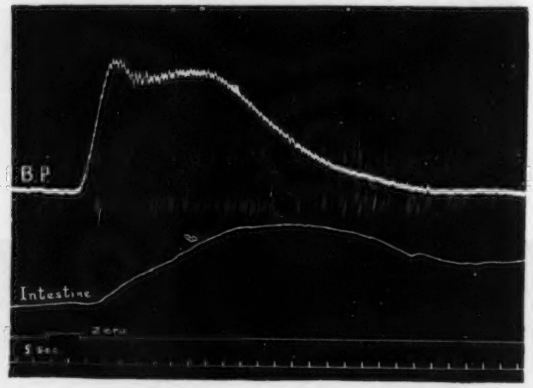

Fig. 10. Dilatation of a perfused loop of intestine of a dog (weight 13.5 kgm.) caused by the injection of 2 cc., 1: 10,000 adrenalin into the jugular vein. Postganglionic fibers intact but all central nervous connection destroyed by cutting the splanchnic fibers. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

had been either passive or else due to stimulation of peripheral structures.

In order to eliminate peripheral effects we perfused loops of intestine by the method already described (1, p. 360). All splanchnic nerve fibers and in some cases the vagi were cut. Six dogs were studied by this method. In every animal adrenalin injected into the jugular vein caused dilatation of the perfused loop of intestine, as great in amount as that usually obtained in perfused loops of which the central nervous connection was intact (fig. 10). In two dogs the dilatation was often preceded by constriction. Cutting the nerves in the stalk of the perfused loop did away with all effects of the injection. Isola-

tion of the collateral ganglia from the central nervous system was verified in each instance by post-mortem dissection. Two cases showed incomplete section of the lesser splanchnics. In the remaining four the destruction of central nervous connection with the ganglia was found to be complete.

If sympathetic ganglia control the adrenalin vasodilatation in the intestine, it is natural to suppose that suitable doses of nicotine should reduce the dilatation by paralyzing the sympathetic nerve cells. This was found to be the case. A cat (3.2 kgm.) was given intravenously a total of 2.1 cc. of a 0.1 per cent nicotine solution divided into four doses. The dilatation in the intestine from adrenalin was smaller in amount after nicotine than before. A second cat (2.6 kgm.) gave a similar result after an intravenous dose of nicotine (1.7 cc. of a 0.1 per cent solution). The intravenous injection in a third animal prevented the intestinal dilatation altogether (fig. 11). In a fourth cat dilatation was prevented by painting the superior mesenteric ganglion with a 1 per cent nicotine solution.

Vasodilatation of the intestine, therefore, is apparently caused by the action of the adrenalin upon some structure in the superior mesenteric ganglion. As an added proof of this we have been able to cause dilatation of the intestine by the direct application of adrenalin solution to the superior mesenteric ganglion. The intestine of a cat was placed in an oncometer. The mesentery was cut and separated from the superior mesenteric ganglion in such a way that a pocket could be made by engaging the cut surface of the mesentery with haemostats, to form a pool of the solution of adrenalin around the ganglion. The solution was simply poured into the pocket and between each application it was washed away with normal saline solution, which was afterwards removed by sponging. The following results were obtained: A small dilatation of the intestine was produced by a 1: 20,000 solution, ten minutes later a 1: 5000 solution produced a more marked dilatation and finally a 1: 1000 solution produced a dilatation which continued to increase over a longer period than the preceding, although the first effects were about the same (fig. 12). In no case was there any appreciable effect upon the blood pressure.

In view of the evidence advanced above, that the dorsal root ganglia contain an adrenalin vasodilator mechanism for the hind limb, it was thought possible that there might be a similar one for the intestine and that this might respond to direct application of a solution of adrenalin. With the results of Bayliss (5) in mind we judged that the

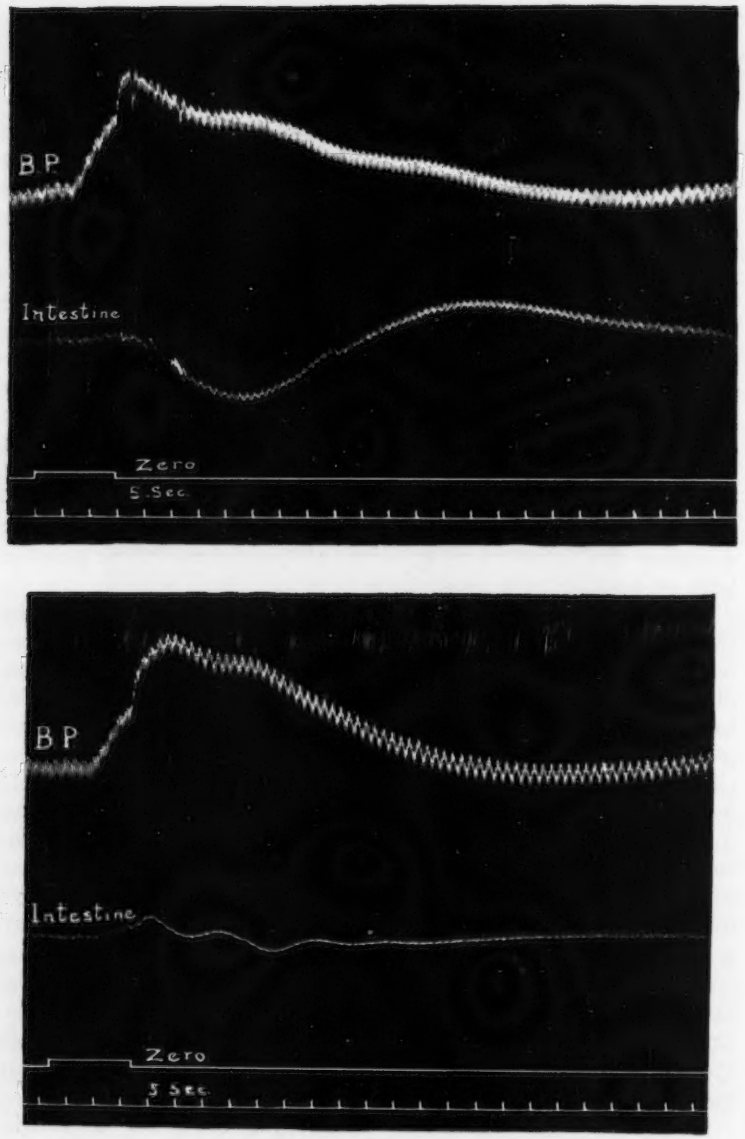

Fig. 11. Failure of intestinal dilatation from adrenalin due to paralysis of the mechanism by nicotine. Upper record, response to 2 cc., 1: 100,000 adrenalin before nicotine. Lower record, response to 2 cc., 1: 100,000 adrenalin after injection of nicotine in the same animal (cat, weight 2.3 kgm.) Base of bellows 20 mm. x 21 mm. (Reduced one-fourth.)

dorsal root ganglia most likely to cause this reaction were those of the twelfth and thirteenth thoracic nerves. We have been able to show that such a mechanism exists, although several of our experi-

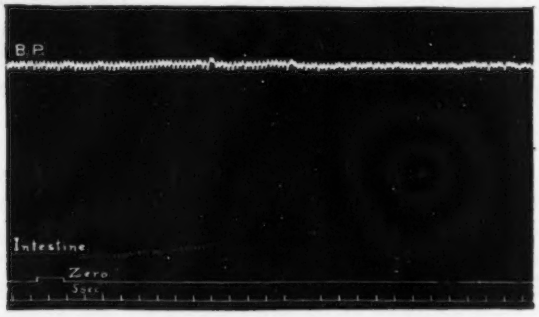

Fig. 12. Dilatation of the intestine due to direct application of 1: 1000 adrenalin to the superior mesenteric ganglion. Cat. Base of bellows 10 mm. x 19 mm. (Reduced one-half.)

ments gave negative results. We investigated seven animals in all, three dogs and four cats, taking records of the volume changes of a loop of intestine on application of a solution of adrenalin 1: 1000 to the ganglia. The preparation of these in the dog was like that described

Fig. 13. Dilatation of the intestine caused by application of 1: 1000 adrenalin to a split dorsal root ganglion. Adrenalin washed away with isotonic salt solution at *X*. Cat. Base of bellows 10 mm. x 19 mm. (Reduced three-fifths.)

above. In the cat we cut the cord across, drew the cut ends back and got access to the ganglia from their central ends, thus avoiding excessive bleeding. In all cases we found it necessary to cut the ganglia

longitudinally to allow the solution free access to the cells. Of the dogs, one showed no change and the two others only slight dilatations, and these not always occurring. In one case constriction took place. Two of the experiments on cats were more successful. In both the application to the ganglia of three or four drops of the adrenalin solution caused a gradual dilatation of the intestine, which was accompanied by no change or by a slight fall in blood pressure and which gradually disappeared after the ganglia had been washed with saline (fig. 13).

DISCUSSION

It is not surprising to find that the central nervous system does not contain the structures stimulated by adrenalin in bringing about vasodilatation. Cannon and Lyman (6) obtained a fall in blood pressure from adrenalin after total destruction of the central nervous system, if ergotoxine had previously been given. Of course it cannot definitely be said that the central nervous system has nothing to do with the problem, since destruction of portions of the brain or cord inhibits or modifies the response, as is evident in the reversal of blood pressure effects. What our results go to show is that the main seat of the reaction is in the sympathetic and dorsal root ganglia. The same conclusion has been arrived at by widely different ways, viz., 1, by perfusion of the organ, together with destruction or removal of the central nervous system and of one or the other set of ganglia, which might be the seat of the reaction; 2, by the destruction of the ganglia in question, thus preventing the dilatation; 3, by the direct application of adrenalin to the ganglia. The fact that to these ganglia is due the greater part of the dilatation caused by adrenalin does not exclude the possibility of some peripheral action on the dilator nerve endings, as various investigators have suggested, notably Gruber in a recent paper (7). Further research on this question is in progress in this laboratory. At present we are not in a position to say whether the gangliar or the peripheral action is more effective in bringing about dilatation.

The nature of the action of adrenalin on the cells of the sympathetic ganglia is still uncertain, whether it is an inhibition of the constrictor elements or a stimulation of a dilator. The first, in the light of the stimulating action of this hormone on the endings of the fibers from these cells seems improbable. In spite of the negative experiments of Bayliss (5) and others we are inclined to attribute our results to a

stimulation of vasodilator cells. If the existence of such cells is a fact, the part, whether cell or synapse, which adrenalin affects, is still uncertain. That it cannot stimulate the fiber directly is evident because no dilatation has ever been obtained in a perfused organ, the ganglia to which have been removed, no matter how few minutes before.

The nature of the adrenalin vasodilator mechanism of the dorsal root ganglia is uncertain, nor is there any evidence as to whether it is similar to that in the sympathetic ganglia. Dogiel (8) has described so-called sympathetic cells in the dorsal root ganglia, but little seems to be known concerning them. Whatever the structure may be, the impulses which are started must be antidromic. From this arises the question of the possible identity of these impulses with those described by Bayliss (9), which brought about dilatation by their action on the vessels of the skin. We have not been able to show conclusively that the dilatation which takes place in a perfused limb, all the nervous connections of which have been destroyed except those with the dorsal root ganglia, is not caused by the vessels of the skin, but all the evidence tends to make it improbable.

SUMMARY

1. Dilatation of the hind limb is brought about by the action of adrenalin on structures located in the sympathetic ganglia of the lower lumbar and sacral regions and in the dorsal root ganglia of the nerves supplying the limb.

2. Dilatation of the intestine is brought about by the action of adrenalin on structures in the superior mesenteric ganglion and in the dorsal root ganglia of the lower thoracic region.

3. Our results tend to support the view that the sympathetic system contains vasodilator fibers to the intestine and to the hind limb.

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XI. THE METABOLIC GRADIENT UNDERLYING INTESTINAL PERISTALSIS

WALTER C. ALVAREZ AND ESTHER STARKWEATHER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication April 1, 1918

For many years physiologists have been teaching their students that food goes down the intestine because of Bayliss and Starling's law (1) or Cannon's myenteric reflex (2). According to this law, a stimulus applied to any part of the gut causes a contraction above and a relaxation below. Interesting and important as this law is, it has a number of limitations which, if better known, would undoubtedly have stimulated investigators to pry into the matter a little further or even to look for a new and more universally applicable law. Cannon himself has pointed out that the myenteric reflex is not always in control and that it "does not govern the rhythmic contractions of the small intestine, the rhythmic peristalsis and antiperistalsis of the colon and probably not the rhythmic waves of the stomach." Since then Gaskell (3) has shown that even the word "reflex" may not be strictly applicable in this connection because recent anatomical studies have made it appear very unlikely that there is any nervous arc over which a true reflex could travel.

On reading the few articles that have been written on the subject, one is struck by the fact that the observers found the reflex hard to demonstrate, often absent, sometimes reversed and always localized within 2 to 3 cm. of the stimulated area. They used traumas and, so far as we know, no one has studied the condition of the muscle above and below a bolus of food. Graphic records of peristaltic rushes obtained by Alvarez (4) with six or seven delicate enterographs rarely showed relaxation before oncoming waves. On the contrary, powerful contractions appeared at some distance in advance, and these often succeeded in stopping the rush. A moment's thought will show the need for this; if the reflex were always active, the bowel would soon be emptied. Food once introduced into the duodenum would never

stop in its rush to the anus. Another thing which suggests that this law is not the last word and that we should look further, is the complaint of the clinician that it has not been of much help to him in that it does not explain the peculiarities of intestinal action in the sick.

Five years ago, while doing some work on the absorption of gas from the bowels of rabbits and cats, Alvarez noticed that there was a great difference between the irritability of the duodenum or jejunum and that of the lower ileum. When 10 cc. of CO_2 or other gas was injected into the ileum, the bowel would respond with a few contractions, after which it would quiet down; the same amount put into the duodenum or jejunum gave rise to active segmentation, which did not cease until the gas had been absorbed or passed on by a peristaltic rush. Feeling convinced that this difference in irritability alone could account for the downward progress of food, Alvarez attempted to measure it in some way. While trying to do this with excised segments beating in Locke's solution, a gradation in rhythmicity was observed (5). Later, graded differences in latent period were found.

Now, the accepted idea until recently has been that the rhythmic contractions are due to stimuli coming from Auerbach's plexus. This view is based on the work of Magnus (6), who found that strips of longitudinal muscle with the nerve-net adherent would contract rhythmically, while strips of circular muscle without the nerve-net would not beat in Locke's solution. Recently, Gunn and Underhill (7) repeated this work, taking great precautions to avoid trauma, and obtained plexus-free circular strips that would contract rhythmically. This is what we should expect from the fact that some segments of intestine will contract better on the second or third day after excision than on the first. One cannot conceive of nerve cells functioning better after that length of time. Moreover, we know now that smooth muscle cells in cultures will contract rhythmically when there is no question as to the absence of nervous tissue (8).

It would seem, then, that the differences in rhythmicity, irritability and latent period must be ascribed to differences in rate of metabolism in the muscles of the different regions. When we remember that we can take a piece of ileum beating ten times per minute and, by warming it, speed up its metabolism so that it will beat fifteen times per minute, it seems probable that the duodenum, which normally beats fifteen times, has a faster metabolism than that of the ileum. A comparison of the coefficients of increase in rate with increase of temperature in different parts of the small intestine lends support to such a view (9).

Experiments with potassium cyanide. We first attacked the problem with a method which has yielded splendid results in the hands of Child (10). He points out that although oxidation is not the only process taking place in the cell, it may serve as a useful index of the total metabolism. It is pretty well accepted that potassium cyanide interferes with oxidative processes and Child has shown that regions with high rates of metabolism and faster oxidation suffer more from the action of weak solutions of this drug than do regions with slower ra es. Thus, if planarian worms of different ages are put into 0.0065 per cent KCN, the younger ones with higher rates of metabolism die before the older ones. If hydras are treated n the same way, those showing the greatest activity die first. In some Ctenophores there is a gradient of susceptibility to KCN in the conducting paths along the rows of swimming plates. The pace-making region suffers so much more than the others that the impulse will sometimes start at the wrong end and the sequence of the beats will be reversed (11).

In doing this work we used five segments of rabbit's intestine in a beaker containing 400 cc. of aerated Locke's solution at 38°C. The animals were killed by a blow on the head and the segments were removed from (1) the upper duodenum (2) the upper jejunum, (3) the upper ileum, (4) the lower ileum and (5) the middle colon. The contractions were all recorded together on the same drum and the segments were all subjected to the same concentration of drug at the same time.

It will be seen from figures 1 and 2 that the addition of 1 part of KCN to 1,300,000 parts of the solution caused a marked loss of tone and rhythmicity in the duodenum and jejunum, while the two segments of ileum were much less affected. We agree with Child that the concentration of the drug must be just right, otherwise the segments will either all stop at once or else they will become acclimated and show nothing. Realizing that a plentiful supply of oxygen might neutralize the effects of the KCN, we stopped aerating the Locke's solution and immediately were able to get better results. Figure 2 shows the marked improvement in the contractions of segments poisoned by KCN when air was passed through the solution. It is plain that the duodenum and jejunum had been suffering much more than had the ileum. Later, when the same dose of KCN was repeated, the first dose still remaining in the beaker, the effects were less pronounced because the oxygen supply was larger.

A large series of experiments was performed next, in which the air current was shut off for varying periods of time, and it was found that with asphyxia alone we could get tracings very similar to those obtained

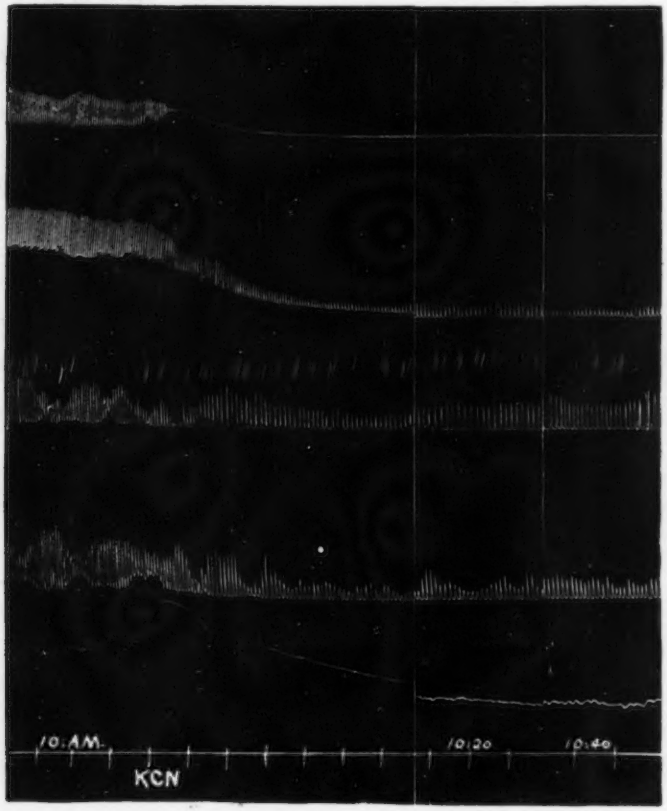

Fig. 1. Graded effects of KCN. From above downwards the segments are from the duodenum, jejunum, upper ileum, lower ileum and colon. Time marking represents minutes.

with KCN. Figures 3 and 4 show how much more the duodenum and jejunum suffered from lack of oxygen than did the ileum. This difference was brought out clearly when air was again allowed to bubble

through the solution. The recovery in amplitude of contraction was then most pronounced in the duodenum.

These results, so comparable with those of Child, furnish considerable proof of the presence of a metabolic gradient from duodenum to

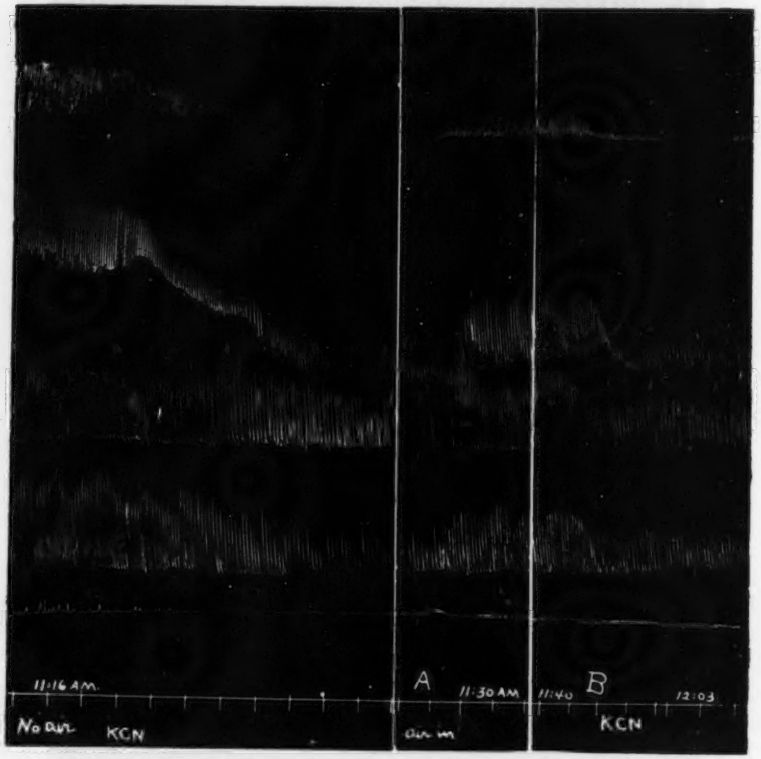

Fig. 2. Effect of KCN on segments in non-oxygenated Locke's solution. At *A*, air was allowed to bubble through the solution; at *B*, more KCN was added.

ileum. The depression observed in the colon is due probably to other causes and not to a rapid rate of metabolism, since most of our other studies indicate that the colonic muscle is even more sluggish than that of the ileum. It will be noted, on turning to the tracings, that KCN has a slowing effect on the rhythm, probably in addition to and apart

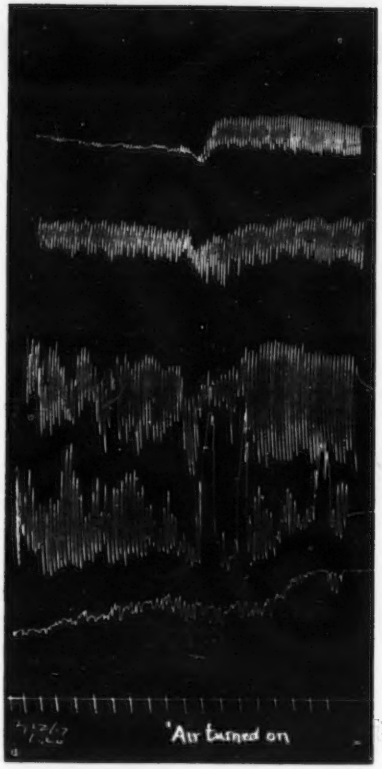

Fig. 3. The effect of asphyxia on segments from different regions.

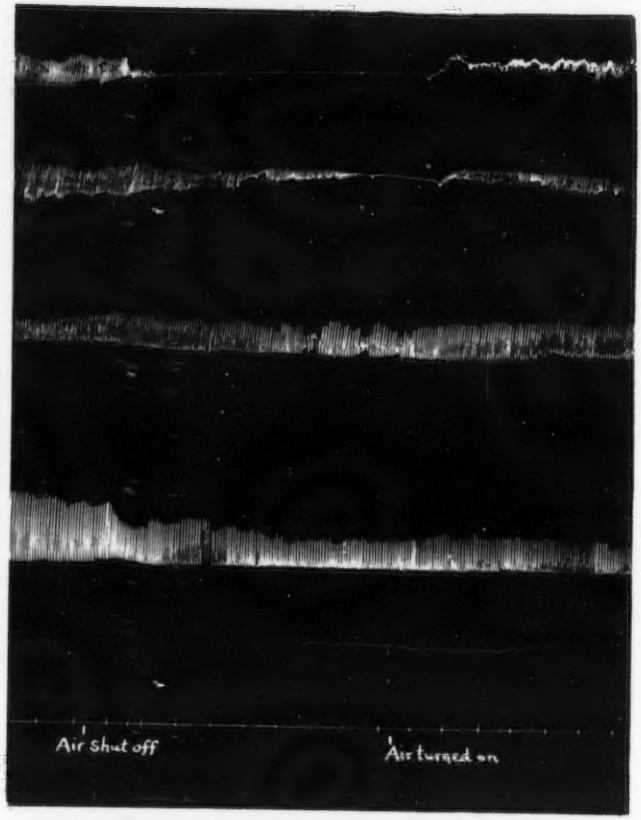

Fig. 4. The effect of asphyxia on segments from different regions.

from its asphyxiating properties. Asphyxia alone does not slow the contractions and although it causes them to disappear in the duodenum, as does KCN, it, unlike KCN, does not affect the colon. In another paper we intend to show that with KCN and other drugs the slowing of the rhythm is most marked in the regions in which the tendency to rhythmic action is weakest. This would explain the marked effect on the colon since those segments are the hardest to start beating in the Locke's solution.

Experiments with adrenalin. The objection may be raised that perhaps five such segments will react to all depressant influences in this graded way and that metabolism has nothing to do with it. After testing some seventy-five drugs, we can say that not only do many of them affect the segments equally but some, such as adrenalin, will often show a beautiful gradient in the opposite direction, that is, the duodenum will be very slightly affected when the ileum is completely paralyzed. This graded effect with adrenalin may easily be due to the same gradiation in intensity of oxidation which we believe produces the differences with KCN. It is well known that adrenalin in dilute solution is rapidly oxidized to an inert substance. If our theory is correct, this change would naturally be brought about soonest in the duodenum and that segment would be the soonest to escape from the influence of the drug. A glance at figure 5 will show that that is what actually happens. With smaller doses, tracings were obtained in which the ileum was seen to suffer considerably in amplitude while the duodenum was unaffected. Apparently the drug was oxidized before it could reach its seat of action in the muscle. Figure 6 shows that these differences are not due to a higher threshold for drug action in the duodenum because immediately following a characteristic adrenalin action, some apocodein produced its most marked effect on the duodenum. In discussing this subject with some physiologists, the objection was raised that these differences should be ascribed to some peculiarity in sympathetic nerve supply. It seems to us that until such complicated differences are clearly understood it is better to rely on the simpler chemical explanation just given.

Measurement of carbon dioxide production. We next attempted to measure the amount of CO_2 given off by the different segments in a unit of time. The technic used was suggested to us by Doctor Van Slyke, to whom our thanks are extended. Weighed segments of rabbit's, cat's and white rat's intestine were put into a large test tube containing Locke's solution at 38°C. In most of the experiments the

0.02 per cent $NaHCO_3$ of the Locke's solution was replaced by 0.01 per cent NaOH although, as was to be expected from the small amount of carbonate present, any error introduced in this way was practically negligible. Oxygen was allowed to bubble through the solution and

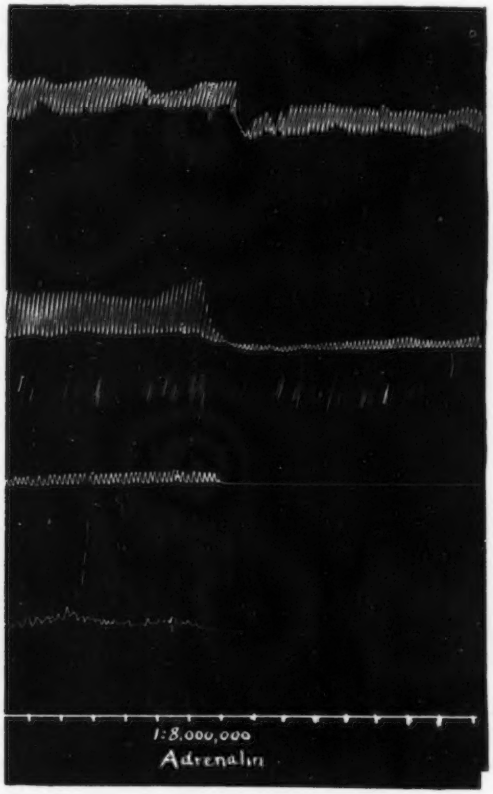

Fig. 5. The graded effect of adrenalin. From above downwards, the segments are from duodenum, upper ileum, lower ileum and colon.

then over through two other tubes, each containing 50 cc. of n/5 $Ba(OH)_2$ solution. We agree with Fletcher (12), who used a similar technic for studies with striated muscle, that these two tubes are sufficient to catch all the CO_2 carried over. At the end of an hour the

two barium hydrate tubes were titrated as rapidly as possible with n/5 HCl. In practically all of these experiments the difference between the CO_2 production of duodenum and ileum was quite marked and was according to expectations. Following are some of the protocols. The figures represent cubic centimeters of n/5 $Ba(OH)_2$ neutralized by CO_2. They vary in the different experiments because different weights and different times were used.

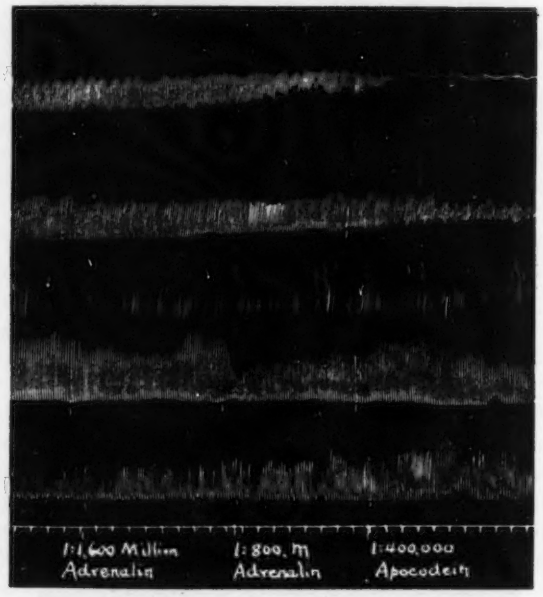

Fig. 6. Graded effects of adrenalin and apocodein. From above downward the segments are from duodenum, jejunum, upper ileum and lower ileum.

	RAT	RAT	RAT	RAT	RAT	CAT
Duodenum	8.5	5.6	9.4	17.4	25.4	20.0
Jejunum				17.0	19.7	15.0
Middle				16.0	10.5	18.0
Ileum	7.8	3.2	7.0	12.0	21.0	15.0

Here, as in all subsequent experiments, poor results were to be expected when some segments contracted more actively or more con-

stantly than others. Good results were obtained either when they all remained quiet or when they all became equally active. It can easily be seen that a very active ileum might give off as much CO_2 as a quiet duodenum. As the segments do not all recover from trauma and start beating at the same time, it was found well in all these experiments to leave the pieces of bowel in warm oxygenated Locke's solution for half an hour after cutting and weighing. When all were going well, they could be transferred gently to the test tubes. Another source of error undoubtedly arises in the fact that so many of the animals have intestinal parasites, i.e., coccidiosus and trichocephalus in the rabbit, round worms and taenias in the cat. Alvarez has pointed out elsewhere that these conditions tend to flatten or even reverse the gradients of rhythmicity and latent period found in normal animals (13). For this reason it is best to give cats and dogs a vermifuge sometime before they are to be used.

Much better results were obtained with the very simple and exceedingly delicate method of Haas (14). Small segments of rat's intestine were opened out, washed thoroughly with Locke's solution, blotted between filter papers and cut to some arbitrary weight, usually 0.4 gram. For purposes of control, two segments were taken from each of the locations described above. After their recovery from this trauma they were transferred to thick, glass Wassermann tubes containing Locke's solution to which 1 part in 20 of a 0.01 per cent watery phenolsulphonephthalein solution was added. The tubes all held about the same amount of solution. They were sealed with corks which had been boiled in paraffin and, in addition, the ends of the tubes, corks and all were paraffined. Only a small bubble of air was left in the tube. These tubes were left in a water bath at 38°C. until the CO_2 formed had brought about a considerable decolorization of the solution. Haas has shown that this method will detect differences in hydrogen ion concentration as small as 1×10^{-5}.

Ordinarily the solution about the duodenal segment would begin to fade within a few minutes and in half an hour the whole tube would often be acid in reaction.

As soon as this decolorization was complete in the tubes containing the duodenum, they were all removed from the bath and arranged against a white background according to the intensity in color. The tubes containing the colonic segments generally retained most of their original pink tint. After the tubes were graded, the labels on the corks were read and put down as in the following protocols. It will

be seen that although at times, probably on account of unequal activity, the controls are somewhat separated, on the whole the gradation is according to expectation. D represents duodenum; J, jejunum; M, middle; I, ileum and C, colon.

Guinea pig

D J D J M M I I C C
D D J M J I I M C C
D J D J M M I I C C
D D J J M M C I I C
D D J J M M I I C C

White rat

D D J J C M M I I C
D D J M J M I I C C
D D J J M I I M C C
D D J J M M I I C C
D D J J C M M I I C

Rabbit

D D J J M I C M I C
J D D J M I I M C C
D D J J M I M I C C
D J M D M J I I C C
D D J J M I M I C C

Cat—muscle alone

D D J C J M M C I I
D D J J M I I M
D J M I C
D J M I C

The results with the white rats and guinea pigs were best, perhaps because their intestines are smaller; longer segments can be used and the proportion of cut surface to segment is much less than in rabbits and cats. It must also be remembered that, with this technic, the Locke's solution is not aerated and we have just seen that lack of oxygen interferes more with the activity of D and J than with that of M, I and C. This difference would tend to obscure the differences in CO_2 production that have been found. The muscle from the cat rarely showed much activity, often none at all.

In order to get some idea of the actual amounts of CO_2 formed, the tubes were opened and titrated rapidly back to the original color with n/200 NaOH. As was to be expected, what with losses of CO_2 and difficulties in getting the end point, the gradation obtained was not so satisfactory as with the closed tubes. In seven experiments, small amounts of the solution were removed and the CO_2 measured in the Van Slyke apparatus. In the following protocol the figures represent cubic centimeters of n/200 NaOH.

Rat (1 hour)

D	D	J	M	J	M	I	I	C	C
5.6	3.8	2.8	2.6	2.4	2.2	1.6	1.2	1.2	1.2

The average amounts in five experiments were

D	J	M	I	C
3.1	2.7	2.5	1.9	1.6

In the following experiments in which the Van Slyke apparatus was used, the figures represent cubic centimeters of CO_2 in 1 cc. of the solution.

Guinea pig

D	J	M	I	C
0.49	0.22	0.18	0.16	0.14

Guinea pig

D	J	D	J	I	I	M	M	C	C
0.30	0.26	0.24	0.21	0.20	0.20	0.17	0.16	0.16	0.02

The average amounts in seven experiments were

D	J	M	I	C
0.30	0.23	0.19	0.18	0.16

The differences between some of the controls in these experiments again show the need for having all of the segments beating well.

The fact that the faded tubes would return to their original color when oxygen was bubbled through them showed that the decolorization was due to CO_2 and not to some other acid.

In order that the bowel might be studied while contracting actively, most of the experiments were done at first with segments containing mucous membrane. As this membrane varies in thickness in different parts of the gut, segments of equal weight might contain different amounts of muscle. An error would creep in also on account of the formation of CO_2 in the mucosa. The fact that decolorization was very slow in tubes containing inactive segments indicates that this error was not large. Moreover, some six experiments done so far show that the CO_2 production in the mucosa is also graded from duodenum to ileum. To rule out all objections, strips of muscle peeled off from the mucous membrane of the cat's intestine were studied with the same methods, and the same graded production of CO_2 was found.

Tests for oxidases and peroxidases. We next attempted to show a gradation in the oxidase and peroxidase content of the muscle from different regions. Preliminary qualitative tests, however, failed to show a measurable amount of these substances in any part of the gut. With or without the addition of H_2O_2, there was practically no bluing of guaiac or benzidin with the crushed muscle. The activity of the reagents was controlled with a little potato juice and with the mucous membrane of the small intestine. Experiments with pyrogallol, hydroquinone, pyrocatechin and metol, using Bunzel's small apparatus (15) showed practically no oxidation on prolonged contact.

There are many reasons why such experiments should be disappointing. We have not as yet identified the ferments, if such there be, which help the tissues to burn their proper fuels,—sugar and fat; and these more or less specialized oxidases which have been identified are probably of lesser importance. Moreover, as Loew has pointed out, the living state of protoplasm seems to be the most important thing in cell oxidations because otherwise, heating to a temperature of 45°, which kills the tissue and does not destroy the oxidases, ought not to interfere with respiration in the way it does.

Catalase estimations. More and more evidence is accumulating to show that the catalase content of a tissue is a better index to its metabolic activity than is its oxidase content. Those who are interested can get an introduction to this literature through the articles of Batelli and Stern (16), Loew (17), Kastle (18), Zieger (19) and Zaleski and Rosenberg (20). In spite of the large amount of work done upon this substance and in spite of the fact that it is found in considerable amounts in most animal and vegetable cells, its biologic significance is still far from clear. Usher and Priestly (21) have shown that in plants H_2O_2 is one of the substances formed during the action of light on CO_2. If not removed immediately by the catalase it bleaches the chlorophyl and puts an end to the photosynthetic reactions. Many believe that catalase has a similar protective function in animal tissues. So far, most of the evidence indicates that it is a highly specific ferment acting on hydrogen peroxide alone. Ewald (22), however, has offered considerable proof for his theory that catalase helps in tissue respiration by loosening the oxygen from oxyhemoglobin. He found that the reduction of oxyhemoglobin in ammonium sulphate solutions takes place faster in the presence of catalase. This is the more suggestive in view of Peters' (23) finding that the amount of oxygen taken up by hemoglobin corresponds to that required to convert its iron into a peroxide. Fischer and Brieger (24) have also shown that the blood oxygen may be held in the form of a peroxide.

It seems probable that in some way the catalase assists in furnishing oxygen to the tissues as fast as they require it. Although there are a number of exceptions still to be explained there is, on the whole, a pretty definite relation between the metabolic activity of a tissue or of an organ and its catalase content. Thus Lesser points out that the largely anaerobic ascaris lumbricoides has one forty-eighth of the catalase content of the aerobic earthworm (25). The catalase content of bacteria has a close relation to their use of oxygen (26). As a general

rule, the sum of the catalase content of blood and liver is less in cold-blooded animals than in warm. There are some exceptions which may be explained by future work. For instance, the blood of a bird has very little catalase although its respiration is very active. This may be due to the derivation of birds from reptilia; and it may be that other tissues make up for the deficiency. Thus it has just been shown that the catalase content of a chicken's skin is higher than that of any other skin studied (27). Other objections have been raised by Amberg and Winternitz (28) who showed that although the fertilization of sea-urchin's eggs leads to an increase of from four to six times their cell oxidation, there is no increase in catalase. Zieger (19), however, showed an increase in catalase content of insects during their metamorphosis to the pupal stage. The conflicting results obtained by some of the workers may easily have been due to their neglect of certain factors modifying the speed of the reaction.

Appleman (29) has shown in potatoes that the catalase content is a better index of respiratory activity than the oxidase content. The catalase rises and falls with the CO_2 production while the oxidase does not. Zaleski and Rosenberg (20) while pointing out a number of objections, conclude from a review of the literature and their experiments with germinating seeds that "the oxidase and catalase effects represent the common function of one and the same substance or complex." Loevenhart and Kastle (30) conclude from parallel experiments with formic acid and H_2O_2 that "in proportion as a substance is able to break down the peroxide, so also is it able to accelerate oxidations." Burge (31) feels sure from some experiments with the muscles of exercised and confined animals that catalase is an index of the metabolic activity of a tissue. Doctor Child permits us to state that work done so far in his laboratory by Mr. MacArthur indicates that the catalase content of different parts of small animals follows the gradients of KCN susceptibility and CO_2 production previously established. Zieger also comments on the fact that younger organisms, with more rapid metabolism, have larger amounts of catalase.

Fortunately, the methods for estimating catalase are very simple. The tissue to be tested is weighed and ground up in a mortar with broken glass. The grinding should be done uniformly or the results will vary. In one experiment the finely ground tissue liberated ten times as much H_2O_2 as did the expressed juice. The glass and tissue are washed into a large test tube with 15 cc. of water, and 15 cc. of 3 per cent hydrogen peroxide is added. This is shaken for fifteen minutes,

and the oxygen given off is measured as it displaces water in a burette. The readings are made at atmospheric pressure. We have found it best to run five tests at a time, using a shaking machine. This not only saves a great deal of time, but it eliminates three considerable sources of error, i.e., differences in the temperature of the room; marked differences in the temperature of the tube, depending on the extent and duration of contact with the shaker's hand; and differences in the amount of shaking. Even with the shaking machine, all the tubes must be held firmly in a block so that some will not be agitated more than others. Another possible source of error avoided by doing the tests together is an unequal lighting. Fortunately, the destructive effect of light on the ferment is slight in the first fifteen minutes, during which time most workers make their determinations. After that, however, surprising differences may be obtained according as the tests are done near to or far from a window (32). When all is ready, the H_2O_2 is run in from a thistle tube, the top of which is connected to one arm of a Y. Another arm connects with the test tube and the other with the burette. Most of our experiments had been done before we read the papers of Loevenhart (33), McGuigan (34), Mendel and Leavenworth (35) and Issajew (36), all emphasizing the importance of neutralizing the hydrogen peroxide before using. We have repeated enough of the work to show that for our purposes it does not make any difference whether this is done or not. The actual amounts of gas given off are larger with the neutral hydrogen peroxide but the gradations to be described later remain the same. The increase is slight for the muscle catalase and more marked with the mucous membrane. To control the technic, a number of tests were run on strips of muscle from the same segment of gut or from adjoining segments, and the error was found ordinarily to be between 1 and 2 per cent.

When segments from different levels were studied, the amounts of oxygen were found to be graded much as the CO_2 was graded. This will be seen from the following figures.

Cat muscle

	PEROXIDE NOT NEUTRALIZED									AVERAGE
Duodenum	21.8	22.5	23.2	22.0	41.0	25.6	39.7	31.7	24.2	27.9
Jejunum...	20.5	20.5	20.3	18.1	35.5	28.0	23.2	24.5	22.7	23.7
Middle.....	17.1	20.2	18.1	16.6	36.7	22.4	26.2	26.7	13.4	21.9
Ileum.....	6.0	13.2	13.5	15.8	34.6	20.5	17.6	18.5	11.7	16.8
Colon.....	9.9	15.0	13.7	18.4	27.8	22.3	19.5	23.3	14.9	18.3

Dog muscle

	H_2O_2 ACID		H_2O_2 NEUTRAL. (PUPPY)		AVERAGE
Duodenum	15.7	14.3	18.3	27.9	19.0
Jejunum	13.2	14.3	17.9	26.6	18.0
Middle	11.9	12.5	13.6	26.0	16.0
Ileum	11.3	12.5	12.6	21.1	14.4
Colon	11.2	11.1	17.0	22.2	15.4

In all these experiments 0.3 gram of tissue was used. The figures represent cubic centimeters of gas evolved after fifteen minutes. In the cat and dog one can easily peel the muscle off the mucous membrane and get it clean except for the thin layer of peritoneum. This cannot be done with the thin bowels of rabbits and rats, so one must there be content with scraping off the mucosa. For some reason or other, this can be done more easily in the duodenum and colon than in the jejunum and ileum.

Rabbit muscle from which the mucosa has been removed by scraping

	H_2O_2 NEUTRALIZED						AVERAGE
Duodenum	42.2	37.2	33.4	36.9	41.5	39.6	38.5
Jejunum	37.8	32.8	27.7	35.6	33.5	32.7	33.4
Middle	39.7	34.2	27.3	24.8	32.4	35.7	32.4
Ileum	32.8	36.7	20.4	31.2	30.0	33.4	30.8
Colon	27.1	29.0	21.4	15.9	21.0	16.8	20.9

It will be seen that the gradient is the same as in the cat. In a number of pregnant and diseased rabbits and in one apparently normal animal, the duodenal figure was lower than that of the jejunum. A similar difference was observed while measuring the latent periods in distempered dogs. The duodenum seems always to be the first to suffer from a general intoxication or from adverse conditions.

As the catalase content of the mucous membrane was found to be graded also from duodenum to colon, the following figures from rabbit and rat may be assumed to represent the sum of graded muscle catalase and graded mucous membrane catalase.

Rabbit muscle with mucosa

	H_2O_2 NOT NEUTRALIZED						AVERAGE
Duodenum	46.8	49.4	47.0	52.8	40.3	51.0	47.9
Jejunum	41.2	44.7	49.8	34.8	38.5	45.5	42.4
Middle	32.3	43.3	44.2	36.2	36.4	39.2	38.6
Ileum	24.8	34.3	39.0	32.4	29.7	19.8	30.0
Colon	14.2	21.9	15.6	21.1	20.8	8.2	16.9

White rat muscle with mucosa

	H_2O_2 NOT NEUTRALIZED						AVERAGE
Duodenum	28.2	18.1	21.2	21.6	30.2	19.5	25.5	23.5
Jejunum	27.2	22.0	13.2	21.2	27.2	19.5	22.2	21.8
Middle	23.3	13.7	13.0	20.8	26.0	19.0	21.4	19.6
Ileum	19.2	12.9	14.0	21.2	26.4	15.7	19.2	18.4
Colon	20.0	6.0	9.8	18.0	22.8	18.3	17.2	16.0

Since in this work the essential thing is the gradient observed in the different sets of five determinations, made at the same time under identical conditions, and not the absolute values of O_2, we have not bothered to reduce the figures to a common temperature and pressure. There are often marked individual differences in the catalase content of muscle from the same region in different animals. The fact that the cat muscle contracts firmly to a pearly white cylinder which does not give the benzidin test shows that the graded differences found are not due to differences in blood content. We reserve for another paper a discussion of similar gradients observed in the catalase content of muscle taken from different parts of stomach and colon. Interesting variations have been found also in the gradient of catalase content in intestines from sick and pregnant animals.

DISCUSSION

It is our belief that the gradient of metabolism shown by these various tests is the underlying basis of downward peristalsis. The impulse in the heart has long been known to follow a gradient of rhythmicity. Miss Hyman, in Child's laboratory, is finding, as was to be expected, that there is a gradient of metabolism underlying the differences in rhythmicity. When we speak of the negativity of the sinus region to

other parts of the heart, we are thinking of the direction of current flow through the galvanometer; on the heart side of the circuit, the sinus region is really positive to other parts, indicating that it has the highest rate of metabolism. Recently Tashiro, with his marvelously sensitive biometer, has demonstrated a gradient of CO_2 production in nerves, and there seems little doubt but that the nerve impulse flows along that gradient (37). In an efferent nerve the gradient is from the

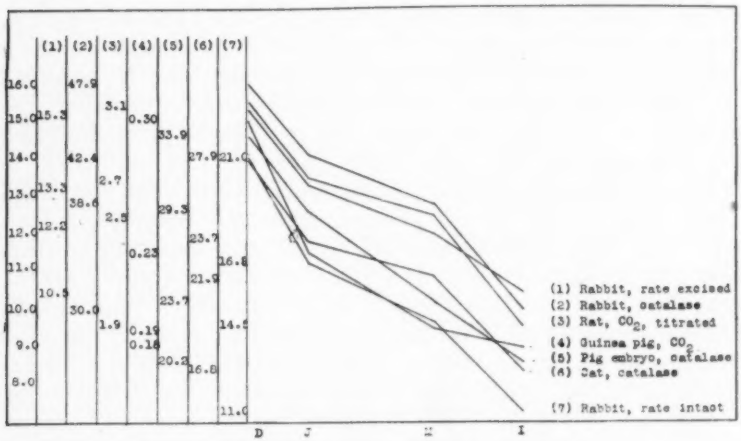

Fig. 7. Showing the parallelism between the gradients of rhythmic contraction, CO_2 production and catalase content in different animals. In order to bring the different curves closer together, an arbitrary set of ordinates was chosen running from 8 to 16. The seven sets of data were then multiplied or divided by factors which would place the first figure for the duodenum between 14 and 16. The original data are shown as ordinates in the seven columns on the left. The numbered columns correspond to the numbered legends identifying the different curves. The abscissae represent the four segments along the gut.

center to the periphery; in an afferent nerve the peripheral end has the greater CO_2 production and the gradient runs toward the center. Similarly we believe that the intestinal contents move aborally because of the aboral gradient of metabolism in the muscle. Scientific men who have been trained by physicists, chemists, electricians and irrigation engineers to expect motion only in the presence of differences in potential or force should find no difficulty in accepting such a theory.

Some may object that we have put the cart before the horse and that a greater amount of CO_2 is formed in the duodenum simply because it beats oftener and does more work. They may point to the fact that poor results were obtained in the CO_2 studies when the segments did not all contract well. To meet this objection we repeated the work with strips of muscle which either did not contract of themselves or else were paralyzed with adrenalin, and we obtained the same graded results. This is what we should expect, also, from the catalase experiments, which show chemical differences in the minced and quiet muscle. Some may still maintain that these differences are brought about by differences in function. Child has aptly compared the life processes to a river whose course is directed by banks which, in their turn, are moulded by the river. We believe that the "banks" just discovered are responsible for the "river;" others may feel that the "river" is still unexplained and that the "banks" are just those which one should expect to find carved by such a stream. It must be remembered, however, that most of the carving takes place over thousands of years during phylogenetic development, and that in any one member of the race these banks are fairly rigid. It seemed well, nevertheless, to follow a suggestion made by Doctor Whipple that we study the intestine in embryos, where function (if any peristalsis attends the formation of meconium, it must be insignificant as compared with that during extrauterine life) has not yet commenced. In terms of Child's simile, let us see whether the "banks" are there before the (individual) "river" has begun to flow.

The following figures represent the catalase value for the muscle (mucous membrane scraped off) from different parts of the intestines of 20 cm. pig fetuses.

	H_2O_2 NEUTRALIZED					AVERAGE
Duodenum	40.6	38.7	28.5	29.9	27.6	33.1
Jejunum	39.9	35.8	20.5	27.2	23.1	29.3
Middle	25.0	25.5	17.2	27.4	23.5	23.7
Ileum	17.0	20.1	16.2	23.6	23.9	20.2
Colon	11.7	15.2	10.6	16.5	18 3	14.5

It will be seen that here again we have the same pronounced gradient from the duodenum to the colon that has been found in adult animals.

Fortunately, we have at our disposal some other experiments which show that the gradient of activity will remain unchanged even when, for many months, the ileum is made to serve as jejunum and the jejunum as ileum. A number of men have reversed long stretches of intestine in dogs and have kept the animals alive for a year or more on a perfectly smooth diet (38). Finally, however, all the dogs died with symptoms of intestinal obstruction, which at autopsy was shown to be due to the accumulation of wisps of straw, bits of bone and other rough material (surreptitiously obtained) just orad to the upper suture. This observation, together with a number of others, convinced the experimenters that the direction of peristalsis had remained unchanged. Although fluids could be forced "uphill" through the reversed bowel, solids could not.

Those who may still feel that the greater activity of the duodenum accounts for its more rapid metabolism have yet to explain the origin of the greater activity. What calls it forth and directs it if it is not some peculiarity of the local musculature? They cannot push the thing off into some ganglion or other because the peculiarities are observed in excised strips of muscle. They can hardly ascribe it to impulses from nerve cells in Auerbach's plexus because the peculiarities persist for three or four days after excision.

If a man designing a cannery wished to convey the cans on a series of belts at varying rates through a number of cooking vats, he would not depend on the intelligence of the cans or upon their desire to linger over some of the processes; he, himself, would regulate the speeds of the different belts to suit the different needs. Similarly, it seems to us that if any one could conceivedly design a bowel in which food and ferments were to be mixed in proper proportions and carried along at rates varying with the needs of absorption, he could hardly do better than to place muscles with a higher metabolic rate, greater rhythmicity, etc., at those points where rapid movement was desired.

These ideas may seem strange to any one who thinks of smooth muscle as an entity, but we feel sure that if such a person were to spend a few months getting records of rhythmic contraction from segments from different parts of the gut, studying the irritability, tone, latent period, form of contraction curve, susceptibility to trauma and disease toxins, and the reactions of rate, rhythmicity and tone to drugs, he would be satisfied that he had been dealing in the different regions with different muscles suited to the different functions. Thus, the feces could not lie quietly in the cecum or colon if the muscles there

were as active and as responsive to stimuli as they are in the duodenum. This brings up another point: that the speed with which the intestinal contents are forwarded depends, probably, not only on the steepness of the gradient but also upon other characteristics of the muscle.

Changes in the gradient of metabolism with symptoms of indigestion might be brought about (1) by a general depression of the body strength or by a general bacterial intoxication which would affect the duodenum more than the ileum; (2) by chronic passive congestion, as in heart disease, the duodenum suffering most from the poor oxygen supply; (3) by a local increase of blood supply, such as probably occurs in the colon in the presence of an inflamed, pregnant or menstruating uterus, and (4) by inflammations, such as appendicitis, which raise the local metabolism above its proper level. In two recent papers, Alvarez (39) has gone over a large number of clinical and radiological observations and has shown how beautifully they fit into such a theory.

Incidentally, those who are interested in the biologic significance of catalase, will find in the parallelism of the curves of rhythmicity, CO_2 production and oxygen liberation in figure 7, added proof of the close relation of this substance to metabolism.

SUMMARY

The "Law of the Intestine" has so many limitations that we should look for an additional or underlying cause for downward peristalsis.

Gradients of rhythmicity, irritability and latent period have been demonstrated in the intestinal muscle from duodenum to colon. All the evidence now points to a myogenic origin for the rhythmic movements.

Five segments of intestine contracting rhythmically in Locke's solution show a graded susceptibility to low concentrations of KCN, the duodenum suffering most. A similar graded effect can be obtained with asphyxia. There is considerable evidence to prove that such gradients of KCN susceptibility and asphyxiation correspond to gradients of oxidative activity.

The graded response of the segments to adrenalin is explained on the same basis. The more rapid oxidation of the drug entering the duodenal wall enables that segment to escape promptly from its action.

Using two different methods, it has been shown that, per unit of weight, there is a graded production of CO_2 both in the muscle and in

the mucous membrane from duodenum to colon. This gradient was observed even when the muscle was kept paralyzed by adrenalin.

No measurable amounts of oxidase or peroxidase could be found in the muscle. A peroxidase is present in the mucous membrane of the small intestine.

The catalase content of muscle and of mucous membrane per unit of weight is found to be graded from duodenum to ileum.

These observations all point to the presence of a metabolic gradient in the muscle, a gradient which the writers believe underlies and gives rise to the gradients of rhythmicity, irritability and latent period. They believe that these gradients determine the direction of peristalsis just as similar gradients direct the impulse in the heart.

Many disease conditions can be explained best on the basis of upsets or differences in steepness in these gradients.

Added proof is given for the view that the catalase content of a tissue is an index to its metabolic activity.

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SECRETIN

III. Its Mode of Action in Producing an Increase in the Number of Corpuscles in the Circulating Blood

ARDREY W. DOWNS and NATHAN B. EDDY

From the Physiological Laboratory of McGill University, Montreal, Canada

Received for publication April 1, 1918

In previous reports (1), (2) we have pointed out that secretin is capable of producing a considerable increase in the number of erythrocytes and leucocytes in the circulating blood. We have further suggested that the increase is due to an increased production of blood corpuscles probably by direct stimulation of both the bone marrow and the lymph glands. If this be true its repeated administration over a long period of time should effect definite changes in the blood picture and, in the organs, the histological changes of increased activity.

In accordance with this idea secretin was given hypodermatically to two rabbits to a total of forty doses. The preparation of secretin employed was a dried acid extract as in our previous experiments (2), and the dose of 10 mgm. per kilogram of body weight was dissolved in 2 cc. of physiological saline solution. To each of two control rabbits 2 cc. of physiological saline solution were administered subcutaneously at the same time and under the same conditions. The injections were made every day for two weeks, three times a week for the next two weeks, then every day for the third two weeks, and finally three times a week for two weeks. Thus the entire time during which the experiments were conducted was eight weeks, from December 10, 1917 to February 4, 1918. Each pair of rabbits comprised a male and a female and for convenience will be referred to hereafter as the secretin rabbits, nos. 1 and 2, and the control rabbits, nos. 3 and 4. They were fed and cared for under our personal supervision. We wish to emphasize particularly that there was at no time evidence of infection in any of the rabbits, nor did either of the females become pregnant. Their appearance remained perfectly normal and all showed satisfactory gain in weight, the average for the four animals being 257 grams during the time under observation.

Daily at first but somewhat less frequently later in the course of the experiments, prior to giving the injections, blood was withdrawn from the ear of each rabbit for counting both the red and white corpuscles. The counts were made early in the morning before the animals were fed. Physiological saline solution and 0.5 per cent acetic acid were used as diluting fluids for the red and white corpuscles respectively and the counts were made in the usual manner with the Thoma-Zeiss apparatus. Blood smears were obtained at the same time, subsequently stained and differential leucocyte counts made, the results of which will be detailed later.

Table 1 gives the initial erythrocyte and leucocyte counts in each of the secretin rabbits made before the first injection and the counts week by week, these latter being in each instance the average of all the counts made on that particular animal during the week. Table 2 gives the corresponding counts for the control rabbits. At the bottom of each table is also given the percentage relation of the various counts, considering the initial count as 100 in each case.

A considerable increase in the leucocyte count was promptly produced in the secretin rabbits. This amounted to 52.52 per cent in the first week; in the third week the increase was 146.69 per cent, and even in the eighth week the total leucocyte count was still 37.56 per cent above the initial count in these rabbits. The control rabbits, meanwhile, showed comparatively slight daily variations in the leucocyte count. On two occasions the weekly average was as much as 20 per cent above the initial count but these high counts were evidently only manifestations of the variation that normally occurs in the number of white corpuscles in the rabbit. The leucocytes in the control rabbits did not show the same great and persistent increase in number that was apparent in the secretin rabbits.

Less pronounced change in the erythrocyte count was effected though a comparison of the two tables permits the observation that, while the initial count in the control rabbits was higher than in the secretin rabbits, at the end of the experiment the count in the secretin rabbits was not only relatively greater, 18.47 per cent, but also absolutely higher. We have observed before that the erythrocyte count in normal rabbits may increase to some extent during long periods under observation probably because the living conditions, food, etc., are improved. The increase in the count in the secretin rabbits, however, is greater and occurred more promptly than any effect we have ever observed in normal animals as the result of environmental improvement alone.

TABLE 1

Erythrocyte and leucocyte counts in secretin rabbits

NUMBER		INITIAL COUNT	FIRST WEEK AVERAGE	SECOND WEEK AVERAGE	THIRD WEEK AVERAGE	FOURTH WEEK AVERAGE	FIFTH WEEK AVERAGE	SIXTH WEEK AVERAGE	SEVENTH WEEK AVERAGE	EIGHTH WEEK AVERAGE
1	W. B. C.	9,300	17,183	22,166	22,266	25,833	29,300	16,250	17,400	13,100
	R. B. C.	6,608,000	5,929,000	4,703,000	6,150,000	5,178,000	6,858,000	6,300,000	6,944,000	7,562,000
2	W. B. C.	10,400	12,866	12,916	26,133	20,333	13,000	14,200	16,950	14,000
	R. B. C.	4,432,000	4,941,000	5,208,000	6,150,000	5,042,000	6,715,000	6,336,000	7,196,000	7,332,000
Averages....	W. B. C.	9,850	15,024	17,541	24,199	22,933	21,150	15,125	17,150	13,550
	R. B. C.	5,520,000	5,435,000	4,955,500	6,150,000	5,110,000	6,786,500	6,318,000	7,070,000	7,447,000
Percentage relations	W. B. C.	100.00	152.52	178.08	246.69	232.82	214.63	153.55	174.03	137.56
	R. B. C.	100.00	98.46	89.77	111.41	92.57	122.94	114.45	128.07	134.90

TABLE 2

Erythrocyte and leucocyte counts in control rabbits

NUMBER		INITIAL COUNT	FIRST WEEK AVERAGE	SECOND WEEK AVERAGE	THIRD WEEK AVERAGE	FOURTH WEEK AVERAGE	FIFTH WEEK AVERAGE	SIXTH WEEK AVERAGE	SEVENTH WEEK AVERAGE	EIGHTH WEEK AVERAGE
3	W. B. C.	7,100	7,833	7,166	11,800	7,833	7,600	6,800	9,000	8,533
	R. B. C.	6,272,000	5,536,000	5,784,000	6,121,000	5,386,000	6,336,000	6,574,000	6,616,000	7,250,000
4	W. B. C.	9,800	9,166	6,916	9,100	9,600	10,100	11,250	12,600	10,433
	R. B. C.	6,176,000	6,081,000	6,194,000	6,253,000	6,377,000	7,530,000	6,856,000	6,481,000	7,244,000
Averages....	W. B. C.	8,450	8,499	7,041	10,450	8,716	8,850	9,025	10,800	9,483
	R. B. C.	6,224,000	5,808,500	5,989,000	6,187,000	5,886,500	6,933,000	6,715,000	6,548,500	7,247,000
Percentage relations	W. B. C.	100.00	100.57	83.31	123.66	103.14	104.73	106.80	127.69	112.22
	R. B. C.	100.00	93.35	96.25	99.44	94.60	111.39	107.89	105.21	116.43

Because the blood picture did not show progressive change during the last two weeks of the experiment, we concluded that maximum effect had been obtained. Accordingly the animals were killed and autopsies performed at this time, the method of procedure, practically that described by Livingston (3), being briefly as follows: Each was killed by illuminating gas and weighed before and after expressing the urine. They were then freely bled by being suspended head downward and the abdomen compressed after cutting both carotid arteries and jugular veins. The alimentary tract from the cardia to the anus was excised, weighed and reweighed after its contents had been expressed. We now had the reduced body weight, i.e., the weight of the animal minus the urine and contents of the gastro-intestinal canal.

In general the gross appearance of the tissues was normal in all four animals. Careful inspection showed absolutely no evidence of any infection having existed. The thyroids, spleen and liver were removed from each rabbit and weighed. These weights expressed in milligrams or grams per kilogram of reduced body weight, together with the data from which the reduced body weight was computed, are given in table 3.

TABLE 3

ANIMAL	NUMBER	WEIGHT WHEN KILLED	WEIGHT MINUS URINE	WEIGHT OF GASTRO-INTESTINAL TRACT AND CONTENTS	WEIGHT OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	REDUCED BODY WEIGHT R. B. W.	WEIGHT OF THYROIDS IN MILLIGRAMS PER KILO OF R. B. W.	WEIGHT OF SPLEEN IN MILLIGRAMS PER KILO OF R. B. W.	WEIGHT OF LIVER IN GRAMS PER KILO OF R. B. W.
Secretin.....	1	1890.0	1883.0	356	207.0	1734.0	138.4	991.9	44.994
	2	2249.0	2242.0	322	179.0	2099.0	94.8	819.4	40.752
	Averages	2069.5	2062.5	339	193.0	1916.5	116.6	905.65	42.873
Control.....	3	2649.0	2649.0	451	304.0	2502.0	86.3	479.6	46.163
	4	1867.0	1844.0	299	179.0	1724.0	64.3	330.6	32.215
	Averages	2258.0	2246.5	375	241 5	2113.0	75.3	405.1	39.189

The thyroids in the secretin rabbits were 54.84 per cent heavier than in the controls, a greater difference than one would expect from individual variations as reported by Livingston (4). Also rabbits 1 and 2 showed slight enlargement of the liver, 9.37 per cent, as compared with the others; and the spleen was more than twice as large as in rabbits 3 and 4, 123.56 per cent.

There was no obvious enlargement of the lymph glands in the secretin rabbits though the cervical and abdominal chains were readily found. Rabbit 3, one of the controls, showed excessive subcutaneous fat, rendering the isolation of lymph nodes in this animal unsatisfactory. Lymph glands from each of the other three were preserved for histological examination.

Finally the head of the tibia in each animal was split open and the cylinder of red marrow carefully removed. In the controls this was of a light pink color, quite soft and very friable. In the others it was considerably darker in color, much more firm and decidedly less friable. These cylinders of marrow were also preserved for histological study. In addition smears of the marrow were made, several from each specimen and as uniform in thickness as possible. These were stained with Wright s stain by the same method employed for blood smears. The difference in the consistency of the marrow from the two pairs of rabbits was very noticeable in the making of the smears. When they were examined microscopically striking differences were observed strongly suggestive of increased activity on the part of the bone marrow of the secretin rabbits.

On the slides from the controls the cells were not numerous being mostly myelocytes with occasional large mononuclear ymphocytes and polymorphonuclear leucocytes. Nucleated red corpuscles were comparatively infrequent. In many fields they were not present and very rarely was more than one seen in a single field. The nuclei of these erythroblasts were deeply stained and quite uniform in appearance. On the other hand, in the smears from the secretin rabbits cells of all types were much more numerous, the most pronounced difference being seen in the nucleated red cells. One or more of these was found in every field and not infrequently as many as six or eight and often more were present in a single field. Furthermore, in most of these the nuclear network was plainly distinguishable and many of these cells were observed which were apparently undergoing cell division presenting various stages of mitosis. Several erythroblasts were also encountered in which the nucleus appeared to be undergoing extrusion. A number of myelocytes were also observed in the process of division. All types of leucocytes were likewise more numerous in these smears.

The absolute increase in the number of cells in one set of slides as contrasted with the other can hardly be attributed solely to unavoidable differences in thickness as the same variation was uniformly shown by all of them. The repeatedly observed evidence of cell division certainly would seem to indicate increased activity.

The cylinders of red marrow and the lymph glands were fixed in Müller's-formalin solution and embedded in celloidin. Sections were cut of a uniform thickness of eight microns and stained with Ehrlich's haematoxylin and alcoholic eosin.

The appearance of the sections of bone marrow from the controls corresponded closely with the usual depiction of normal red marrow, consisting of a rather loose network of cells with large spaces probably previously filled with fat. In the other sections the supporting reticulum could with some difficulty be made out but the spaces were closely packed with cells. These were chiefly myelocytes and erythroblasts with the former predominating. Only rarely small vacuo es were seen, fat cells probably. The myelocytes and erythroblasts, as in the smears, presented evidence of cell division. The absolute number of non-nucleated red corpuscles was greater in the secretin sections than in the controls. There can therefore be no doubt that the bone marrow was much more active in the rabbits which had been given secretin than in those to whom saline had been administered.

In the case of the lymph glands the evidence of increased activity was less striking. While the glands grossly were not obviously enlarged they were very readily found in the secretin rabbits even though no. 2 was quite as fat as no. 3 of the controls in which we were unable to isolate any glands satisfactorily for sectioning. The lymph gland sections showed the cells more closely packed in the glands from the secretin rabbits than in those from the controls. In the former the cells almost overlapped in some cases, whereas in the latter they were surrounded by free spaces at least as wide as the cells and usually wider.

The number of white corpuscles in the circulating blood of the secretin rabbits would seem to be directly proportional to their increased production, but the evidence of increased production of red corpuscles far surpasses the increase in the erythrocyte count. For this reason the question naturally presents itself: If such greatly increased activity of the bone marrow is produced by secretin, why is there not a greater and more persistent increase in the number of erythrocytes in the circulating blood? A clue to the answer to this question would seem to be afforded by the enlargement of the liver and spleen. According to Robertson and Rous (5) overactivity on the part of the bone marrow results in the production of immature erythrocytes whose resistance to disintegration in the blood stream is below normal. The remains of these corpuscles which have gone to pieces throughout the circulation

are removed from the blood chiefly by the spleen but partly also by the liver. The accumulation of this debris, according to the same authors, is the chief cause of the enlarged spleen in anaemias. Possibly we have a similar condition brought about by the repeated administration of secretin, which is obviously producing overstimulation and therefore conceivably causing the production of less perfect corpuscles which undergo disintegration in the blood stream and are removed by the spleen and liver.

Another explanation of the apparent discrepancy between the production of the red corpuscles and the number in circulation is also to be found in the activity of the liver. It has been repeatedly demonstrated that secretin stimulates the secretion of bile (6), (7). Possibly this increased production of bile requires and brings about an increased destruction of the red corpuscles which is only a little more than offset by their increased production. Here again the enlargement of the spleen would have to be explained by accumulation in it of fragmented corpuscles. A direct relation between the disintegration of the red corpuscles with the liberation of haemoglobin and the secretion of bile pigment is claimed by Eppinger and Charnas (8), Wilbur and Addis (9) but denied in the more recent work of Whipple and Hooper (10).

Further evidence of the production of new corpuscles in response to secretin can be adduced from the study of the blood smears. The material for this study was obtained coincidentally with the making of the leucocyte counts in the experiments recorded in a previous paper (2) and, as previously mentioned, from the animals used in the preparation of the present report. The blood smears were stained according to the method recommended by Russell (11), viz., Wright's stain, 2 minutes; water, 5 minutes; dilute Manson's stain, 40 seconds; washed and dried. In every case at least two hundred cells were counted for deriving the percentages and the usual number counted was three hundred. Ehrlich's classification of the white corpuscles has been followed simply because it is so widely known.

We have made altogether sixteen determinations of the differential leucocyte count in apparently normal rabbits as they came to us before they were subjected to any experimental procedure. An average of the sixteen determinations gives us the following figures: Total count, 10,372 white corpuscles per cubic millimeter of blood; small mononuclear lymphocytes, 7.5 per cent; large mononuclear lymphocytes, 13.3 per cent; transitional leucocytes, 5.4 per cent; polymorphonuclear

neutrophilic leucocytes, 69.7 per cent; polymorphonuclear eosinophilic leucocytes, 3.6 per cent; polymorphonuclear basophilic leucocytes, 0.5 per cent (table 4).

Differential leucocyte counts were also made from smears obtained at the time of maximum count in ten experiments in each of which the rabbit had been given subcutaneously a single dose of 1 cc. of secretin solution (10 mgm. of the dried acid extract) per kilogram of body weight. The details of these experiments have been recorded pre-

TABLE 4

Differential leucocyte counts in normal rabbits

NUMBER	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
1	9,300	14.2	11.0	6.8	63.5	4.0	0.5
2	10,400	10.3	15.0	6.0	66.0	2.0	0.7
3	7,100	6.0	16.0	6.0	68.0	3.0	1.0
4	9,800	9.0	12.0	5.0	69.7	4.0	0.3
5 (1)*	4,800	7.0	13.3	4.5	67.6	7.3	0.3
6 (2)	10,400	7.0	9.0	5.0	74.0	4.7	0.3
7 (3)	9,600	5.0	15.0	6.0	69.0	4.5	0.5
8 (4)	6,200	2.7	10.7	5.6	77.0	3.5	0.5
9 (5)	11,600	10.3	11.3	3.0	72.0	3.0	0.4
10 (6)	20,000	8.0	13.5	4.0	70.0	4.0	0.5
11 (7)	15,600	10.0	13.5	6.0	67.0	3.0	0.5
12 (8)	12,654	4.0	17.0	6.0	70.5	2.0	0.5
13 (9)	10,900	7.0	18.0	6.0	65.5	3.0	0.5
14 (10)	7,400	8.5	10.5	5.5	73.0	2.0	0.5
15 (15)	11,000	5.0	15.0	6.0	70.0	3.5	0.5
16 (16)	9,200	6.0	12.0	5.0	72.4	4.1	0.5
Averages.....	10,372	7.5	13.3	5.4	69.7	3.6	0.5

* In this and succeeding tables the bracketed figures are the experiment numbers of previous report (2).

viously (2). Averaging these ten counts we get the following figures: Total count, 15,113 white corpuscles per cubic millimeter, which was an average increase of 44.2 per cent as compared with the initial counts in the same ten experiments; small mononuclears, 14.01 per cent; large mononuclears, 14.28 per cent; transitionals, 4.3 per cent; polymorphonuclear neutrophiles, 64.55 per cent; polymorphonuclear eosinophiles, 2.42 per cent; polymorphonuclear basophiles, 0.44 per cent (table 5).

TABLE 5

Differential leucocyte counts at time of maximum effect following single dose of secretin

NUMBER	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
5 (1)	7,800	16.0	6.0	3.0	71.0	3.0	1.0
6 (2)	13,600	17.4	9.0	4.0	67.0	2.3	0.3
7 (3)	11,250	21.0	6.0	3.0	67.0	2.5	0.5
8 (4)	14,200	10.0	11.0	4.5	71.0	3.0	0.5
9 (5)	16,600	21.0	13.0	3.0	60.0	2.7	0.3
10 (6)	34,800	12.0	21.0	5.0	60.0	2.0	0.0
11 (7)	11,786	9.3	19.3	6.0	62.0	2.7	0.3
12 (8)	15,800	8.0	20.0	5.0	64.5	2.0	0.5
13 (9)	13,100	13.0	21.0	5.0	58.5	2.0	0.5
14 (10)	12,200	12.0	16.5	4.5	64.5	2.0	0.5
Averages.....	15,113	14.01	14.28	4.3	64.55	2.42	0.44

Following the administration of secretin, therefore, there is an absolute increase in all varieties of the white corpuscles, a considerable relative increase in the number of the small mononuclear lymphocytes and a slight relative increase in the large mononuclear lymphocytes, with a relative diminution in the polymorphonuclear leucocytes.

Tables 6 and 7 give the total and differential leucocyte counts in two experiments, also previously recorded in full (2), in each of which 1 cc. of secretin solution (10 mgm. of the dried acid extract) per kilogram of body weight was injected subcutaneously at hourly intervals for three doses. Here again there is a relative increase in the mono-

TABLE 6

Experiment 15 (15)

TIME	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
Initial.....	11,000	5.0	15.0	6.0	70.0	3.5	0.5
1st hour.....	13,300	10.0	17.5	4.0	65.5	2.5	0.5
2d hour.....	11,600	10.5	16.0	5.0	63.5	4.0	1.0
3d hour.....	11,400	15.0	11.0	3.0	66.0	4.0	1.0
5th hour.....	14,600	7.5	10.0	3.0	75.5	3.5	0.5
6th hour.....	11,800	3.0	7.5	3.5	82.0	3.5	0.5

TABLE 7

Experiment 16 (16)

TIME	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
Initial	9,200	6.0	12.0	5.0	72.5	4.0	0.5
1st hour	15,300	10.0	16.0	5.0	65.0	3.5	0.5
2d hour	13,200	7.7	17.0	5.3	65.7	4.0	0.3
3d hour	12,600	7.0	17.0	4.5	67.5	3.5	0.5
5th hour	16,200	3.5	12.5	3.0	76.5	4.0	0.5
6th hour	12,200	3.0	9.0	4.5	79.0	4.0	0.5

nuclear lymphocytes. Toward the end of the experiment the opposite condition prevails, viz., a relative increase in the polymorphonuclear leucocytes with a relative diminution in the lymphocytes, persisting even after the falling off of the total count Moreover, we five times observed nucleated red corpuscles in the blood smears of this series, in each instance in a smear obtained after the administration of the third dose of secretin.

We have further recorded the total and differential counts in each of the four rabbits of the present series at irregular intervals throughout the course of the experiment. These figures are given in tables 8, 9, 10 and 11. In this case the secretin rabbits show a slight relative increase in the large mononuclear lymphocytes and also the transitionals with a relative decrease in the small mononuclear lymphocytes and with practically no change in the proportion of the polymorphonuclear leucocytes. For example, averaging the initial counts we get: Total count, 9,850; small mononuclears, 12.25 per cent; large mononuclears, 13.0 per cent; transitionals, 6.5 per cent; polymorphonuclear neutrophiles, 64.75 per cent; polymorphonuclear eosinophiles, 3.0 per cent; polymorphonuclear basophiles, 0.5 per cent; and averaging all the counts made after administration of secretin we get: Total count, 16,300; small mononuclears, 9.37 per cent; large mononuclears, 15.2 per cent; transitionals, 9.56 per cent; polymorphonuclear neutrophiles, 62.12 per cent; polymorphonuclear eosinophiles, 3.0 per cent; polymorphonuclear basophiles, 0.5 per cent. A comparison of the differential counts of the control rabbits fails to show similar variations in the relative proportions of the different types of white corpuscles.

TABLE 8

Differential leucocyte counts in secretin rabbit, no. 1

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	9,300	14.0	11.0	7.0	63.5	4.0	0.5
December 19, 1917....	19,600	14.5	9.5	10.5	63.0	2.0	0.5
December 31, 1917....	15,600	10.0	10.0	8.0	68.5	3.0	0.5
January 26, 1918....	18,000	7.0	17.5	10.0	62.0	3.0	0.5
February 2, 1918....	15,400	8.0	17.0	9.0	62.5	3.0	0.5

TABLE 9

Differential leucocyte counts in secretin rabbit, no. 2

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	10,400	10.5	15.0	6.0	66.0	2.0	0.5
December 19, 1917....	11,600	9.0	16.0	8.0	63.0	2.5	0.5
December 31, 1917....	20,800	10.0	18.0	10.0	57.0	4.0	0.5
January 26, 1918....	15,200	7.5	17.5	11.0	59.5	4.0	0.5
February 2, 1918....	14,200	9.0	16.0	10.0	61.5	3.0	0.5

TABLE 10

Differential leucocyte counts in control rabbit, no. 3

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	7,100	6.0	16.0	6.0	68.0	3.0	1.0
December 19, 1917....	9,200	6.0	17.0	6.0	67.0	3.7	0.3
December 31, 1917....	7,400	5.0	19.0	5.0	66.3	4.0	0.7
January 26, 1918....	10,800	7.0	14.0	5.0	70.5	3.0	0.5
February 2, 1918....	9,200	5.0	16.0	5.0	69.5	4.0	0.5

TABLE 11

Differential leucocyte counts in control rabbit, no. 4

DATE OF OBSERVATION	TOTAL COUNT	LYMPHOCYTES		LEUCOCYTES			
		Small	Large	Transitional	Neutrophile	Eosinophile	Basophile
December 10, 1917....	9,800	9.0	12.0	5.0	69.7	4.0	0.3
December 19, 1917....	6,600	6.0	17.0	6.0	66.5	4.0	0.5
December 31, 1917....	8,200	10.5	15.0	6.0	65.0	3.0	0.5
January 26, 1918....	13,200	8.0	12.0	6.0	69.5	4.0	0.5
February 2, 1918....	10,400	8.0	16.0	5.0	66.5	4.0	0.5

Such alterations in the relative percentages of the different forms of leucocytes, as have been recorded in tables 4 to 11 inclusive, where there is an absolute increase in the total white corpuscle count, is presumptive evidence of the formation of new cells especially of the types relatively increased.

SUMMARY

Therefore, we conclude that the increase in the number of red and white corpuscles per cubic millimeter of circulating blood shown to take place in the rabbit after the administration of secretin is dependent upon increased production of new blood cells. This greater production is apparently due to stimulation of the bone marrow and lymph glands by secretin. The evidence on which this conclusion is based is: the autopsy findings, the changes in the smears of bone marrow, the histological alteration in both the bone marrow and the lymph glands, the variation in the relative proportions of the white corpuscles and the appearance of nucleated red corpuscles in the circulating blood.

We wish to acknowledge the assistance rendered by Messrs. L. and M. Notkin in the conduct of these experiments.

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II. FURTHER STUDIES ON THE RESPONSE OF THE VASOMOTOR MECHANISM TO REFLEX AFFERENT NERVE STIMULATION

C. M. GRUBER AND O. S. KRETSCHMER

From the Laboratory of Physiology in the University of Colorado and the Henry S. Denison Research Laboratory. *With the aid of the Elizabeth Thompson Science Fund*

Received for publication April 2, 1918

It has recently been established that a slow rate of stimulation and a weak current favor the depressor responses, and a rapid rate of stimulation and a strong current favor the pressor responses of the vasomotor mechanism. In this connection we shall restrict ourselves to the discussion of the literature directly pertaining to the subject, and refer the reader to more general discussions in recent articles by Gruber (1) and by Hunt (2).

Gruber (1) demonstrated that the rate of the stimulation as well as the strength of the stimulus plays an important part in determining whether a reflex vasodilator response or reflex vasoconstrictor response will result upon stimulation of cut afferent nerves. He noted that slow rates of stimuli, 1 to 10 per second, were favorable for the reflex depressor response, whereas rapid rates, 15 to 20 per second, were favorable in the production of the reflex pressor response. These results have been confirmed by Hunt (2). He cites an experiment in which a "tetanizing" current (about 80 interruptions per second) produced a rise of 15 mm. of mercury. The same strength of current interrupted 6 times per second produced a fall of 14 mm. of mercury. In 1895 he explained the differences, fall and rise, in blood pressure obtained upon stimulating a cut afferent nerve on the hypothesis that there are present in the same nerve trunk two separate sets of afferent fibers—vasodilator and vasoconstrictor (3). To meet the differences in thresholds determined by Martin and Lacey (4), 7.5 for the depressor reflex and 280 Z units for the pressor reflex, Ranson and Billingsley (5) advanced the hypothesis that the same nerve fiber is connected with a vasodilator center and a vasoconstrictor center, and that the resultant effect of stimulation is determined by the difference in irritability

of the two, i.e., a weak stimulus reaches or excites the dilator more readily than it does the constrictor center. According to Ranson and Billingsley, the "depressor path" is through a long fiber tract with few relays, whereas the "pressor path" is through a series of short relays.

Vincent and Cameron (6) claim that the fall in blood pressure produced upon the stimulation of cut afferent nerves is atypical and that the rise in pressure is the natural response. They attribute the fall in pressure largely to changes in respiration, i.e., "hyperrespiration," and believe it to be the result of (1) mechanical interference with the heart's action; (2) mechanical interference with the return of the blood to the heart. They report that the extent of the fall of pressure appears to be largely proportional to the violence of the respiratory activity. They say, "Sometimes when the respiration is very violent, a pure fall of blood pressure may take place (see fig. 5)," (pp. 54–64–77).

In their work they could not always satisfy themselves as to the different effects of weak and strong currents produced by sliding the secondary coil away from the primary coil. On several occasions, however, they were able to obtain a fall in pressure with a weak current and a rise with a strong current by changing the number of cells in the primary circuit. They say,

> This effect is interpreted by Reid Hunt as pointing to the existence of "depressor or reflex vasodilator fibres" in the sciatic and similar nerves. If this is the case, opening the thorax should not alter the qualitative result. So far we have been unable to notice the difference just recorded between weak and strong currents respectively when the thoracic cavity has been opened.

They failed to state the rate of interruption of the primary current. However, we presume from a study of their results that it was a tetanizing current. As has been said, this rate of interruption favors the production of the reflex vasoconstrictor rather than the reflex vasodilator response.

In the light of some of the previous work done by one of us (1) upon the vasomotor system, we could not reconcile ourselves to the idea that the fall in blood pressure could have been influenced in the least by the respiratory movements affecting the heart's activity. (See *1*, fig. 3.)

The present research was therefore undertaken to determine to what extent the fall in blood pressure could be due to respiratory interference and incidentally to determine the approximate thresholds for the depressor and pressor responses in dogs with a given rate of stimulation.

METHOD

Both dogs and cats were used in these experiments, in all cases with light ether anaesthesia. The skin was incised on the median line of the neck and the animal tracheotomized. The blood pressure was always registered from the left carotid artery. A signal magnet, which marked intervals of five seconds, was placed at the atmospheric pressure line of the manometer.

For reflex afferent stimulation, the saphenous, femoral, ulnar and peroneal nerves were used. Each nerve, as used, was isolated, cut and the central end fastened in a Sherrington shielded electrode (7). The nerve was kept warm and from being stretched when the secondary coil was moved by fastening the two flaps of skin snugly on either side of the electrode with paper clips.

The stimulating current was usually 0.1 ampere in the primary circuit, and the strength of the secondary current was determined in Z units according to the Martin method (8). In a few cases, 0.5 and 1 ampere in the primary current was used.

The rate of stimulation, with the exception of a few cases in which it was 23 per second, was 7 interruptions per second. No attempt was made to short circuit the make shocks as it was thought that this alternating effect would overcome any polarization which might take place in the nerve trunk at the point of stimulation. The method of interrupting the current was the same as that employed in a previous research by one of us (1).

The effect of central nerve stimulation was tested both before and after the opening of the thorax in the experiments on dogs. In the experiments upon cats it was thought unnecessary to stimulate the nerve before the thorax was open, on account of the uniformity of previous results.

In all the experiments the ribs were transected on the side of the thorax and the entire sternum and parts of the ribs attached to it removed. This completely exposed the heart and lungs. Artificial respiration was carried on by a motor interrupting an air blast. Care was taken to maintain as nearly as possible a body temperature of 38°C. This was successfully done by placing an electric lamp over the animal.

RESULTS

The threshold stimulus. The threshold stimulus for the depressor response varied in the seven readings on dogs with the thorax closed from 4.2 to 20 Z units, or an average of 8.3 Z units. The stimulus necessary to bring about the pressor response varied in the same animals from 220 to 2425, or an average of 628 Z units.

Readings were made on animals with the thorax opened. The threshold for the depressor response varied in these from 4.2 to 16.8, with an average of 7.3 Z units, and for the pressor response 59 to 3100, with an average of 844.8 Z units. (See table 1.)

TABLE 1

The approximate threshold stimuli for pressor and depressor responses in dogs. Rate of stimulation 7 times per second

THORAX CLOSED			THORAX OPENED		
Nerve	Approximate threshold fall in Z units	Approximate threshold rise in Z units	Nerve	Approximate threshold fall in Z units	Approximate threshold rise in Z units
Left saphenous....	4.6	485	Left saphenous.....	4.2	295
Right saphenous...	4.2	485	Femoral.....	4.2	220
Ulnar.....	20.3	485	Ulnar.....	16.8	220
Right saphenous...	4.2	220	Peroneal.....	4.2	59
Right saphenous...	4.2	485	Femoral.....	4.2	1100
Ulnar.....	16.8	220	Left saphenous.....	4.2	3100
Saphenous.....	4.2	2425	Peroneal.....	4.2	2425
			Saphenous.....	7.5	485
			Peroneal.....	16.8	59
			Saphenous.....	7.5	485
Average.....	8.3	628		7.3	844

The average for the depressor response in these experiments upon dogs is the same as that obtained by Martin and Lacey (4) in experiments on decerebrate cats but lower by 40 per cent than the results they obtained with ether or urethane anaesthesia on the same experimental animal. This difference is probably due to the difference in the depth of anaesthesia.

The average current strengths of 844 and 628 which we found necessary to produce a rise in blood pressure are much higher than that found by Martin and Lacey, 280 Z units.

Optimum strength of stimulus. With a rate of stimulation of 7 per second the maximal fall in blood pressure was obtained in 80 per cent of the experiments with a current of 16.8 Z units in the animals with the thorax closed or opened. The average fall in blood pressure with the thorax closed, tested upon 5 dogs and 7 nerves, was 15 mm. of mercury, or a fall of 9.5 per cent. The average fall in blood pressure tested upon eleven different nerves in five different animals with the thorax opened was 13.3 mm. of mercury, or a fall of 10.9 per cent. (See table 2.)

TABLE 2

The maximal fall in blood pressure in millimeters of mercury, and the fall in blood pressure in per cent, upon reflex afferent nerve stimulation in dogs. Rate of stimulation 7 per second

THORAX CLOSED				THORAX OPENED			
Nerve	Strength of current in Z units	Maximal fall in blood pressure in millimeters of Hg.	Fall in blood pressure in per cent	Nerve	Strength of current in Z units	Maximal fall in blood pressure in millimeters of Hg.	Fall in blood pressure in per cent
Saphenous	16.8	25	16.6	Saphenous	16.8	10.0	8.0
Saphenous	16.8	14	9.0	Peroneal	16.8	10.0	8.0
Ulnar	175.0	6	4.0	Ulnar	59	13.0	9.0
Saphenous	7.5	7	4.4	Ulnar	7.5	10.0	9.0
Saphenous	16.8	14	8.5	Femoral	16.8	21.0	16.0
Ulnar	7.5	8	5.0	Saphenous	16.8	20.0	14.5
Saphenous	16.8	31	19.0	Peroneal	16.8	18.0	13.0
				Saphenous	16.8	12.0	10.0
				Peroneal	16.8	8.0	10.0
				Saphenous	16.8	8.0	7.0
				Femoral	485	20.0	16.0
Average		15	9.5			13 3	10.9

That the depressor response is as readily elicited in dogs as we have previously shown it to be in cats can be seen in figure 1. The animal was under light ether anaesthesia. The rate of interruption of the stimulating current was 7 per second. The maximal fall was obtained at *3*, in which the strength of stimulus was 16.8 Z units. There occurred with this strength of stimulus applied to the saphenous nerve a drop in blood pressure from 150 to 126 mm. of mercury, a fall of

16 per cent. No noticeable change in respiration occurred during the experiment.

Figures 2 and 3 are records taken from the same animal as was figure 1, but with the thorax opened. The opening measured 4 by 6 inches. Upon stimulation of the femoral nerve in figure 2 the blood

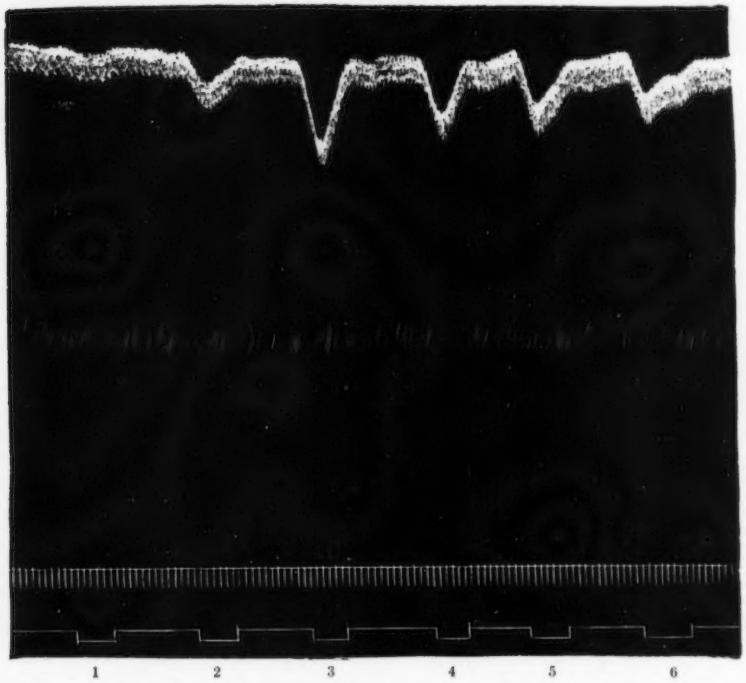

Fig. 1. Dog. Thorax closed. Saphenous nerve. In this and the following records, the upper curve is that of blood pressure, the middle zero presure, and time in 5 seconds, and the lower line the time and duration of stimulation. Rate of stimulation, 7 per second. Strength of stimulus in Z units at *1*, 4.6; *2*, 7.5; *3*, 16.8; *4*, 16.8; *5*, 25; *6*, 42.5.

pressure decreased from 135 to 118 mm. of mercury, or a fall of 12.6 per cent at *2* with the same strength of stimulus as in figure 1, *3*.

That the opposite response can be obtained by increasing the strength of stimulus in animals with the thorax opened and with heart and lungs entirely exposed, is demonstrated in figure 3. At points *1*, *2* and *3*

electrical excitation of the peroneal nerve produced pure falls in blood pressure. At *4* the reversal took place with a current strength of 59 Z units. In only one other case were we able to produce a rise in blood pressure with so weak a current. In all other nerves in which such a response could be obtained the strength of current necessary to bring about the reversal was 220 or more Z units. In a number of nerves we were unable to get the pressor response with any strength of current

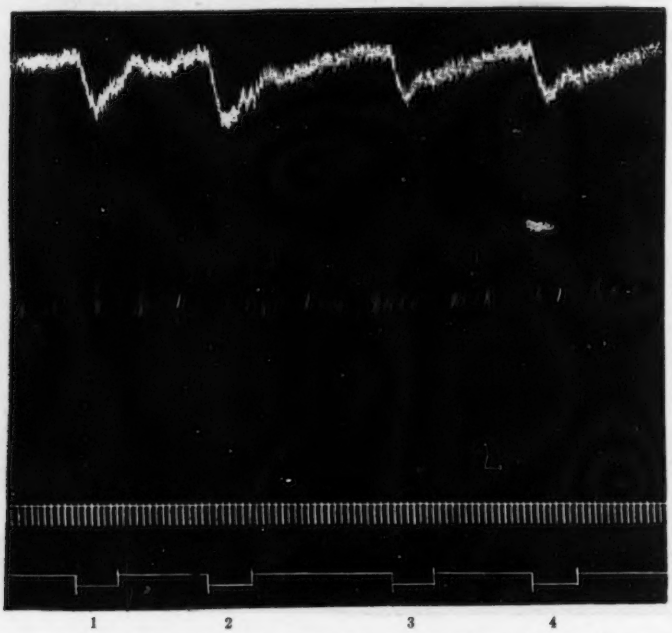

Fig. 2. Dog. Thorax opened. Femoral nerve. Strength of stimulus in Z units, *1*, 7.5; *2*, 16.8; *3*, 59.0; *4*, 175.5.

available. Figure 4 is presented to show that even very strong currents interrupted 7 times per second do not produce a rise in blood pressure. The femoral nerve was stimulated at *1* with a current of 485, at *2* with 2425 and at *3* with 4850 Z units. The blood pressure fell in each case and at *1*, 20 mm., or a decrease of 16.6 per cent.

Similar curves were obtained from the cats used in these experiments. With a rate of stimulation of 7 per second we never failed to

obtain a fall in blood pressure in animals with the chest closed or opened. The extent of the fall was modified by the depth of anaesthesia; the lighter the anaesthesia, the lower the threshold and the greater the extent of decrease.

The respiratory rôle. Our experiments do not support Vincent and Cameron's theory that the fall in blood pressure is brought about by movements of respiration which interfere with the heart's activity.

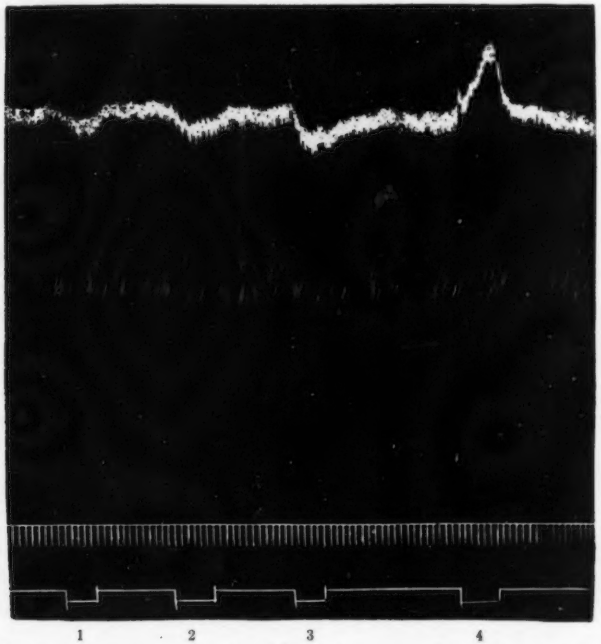

Fig. 3. Dog. Thorax opened. Peroneal nerve. Strength of stimulus in Z units, *1*, 4.2; *2*, 7.5; *3*, 16.8; *4*, 59.

They found that as the thorax was opened, the fall in blood pressure disappeared. They were unable to obtain a difference in the response of the vasomotor system to weak and strong stimuli when the thorax was opened. Only a rise was obtainable.

We found that not only was it possible to obtain a fall in blood pressure with the thorax opened, but that the threshold strength of stimulus was approximately the same in either case. (See table 1.)

The results in table 2 show that it is not only possible to obtain a fall in blood pressure independently of respiratory excitation and the resultant interference with the heart's activity, but that the extent of this fall is greater for animals with the thorax opened.

The differences observed between Vincent and Cameron's results and ours are in all probability due to differences in the rate of interruption of the stimulating current.

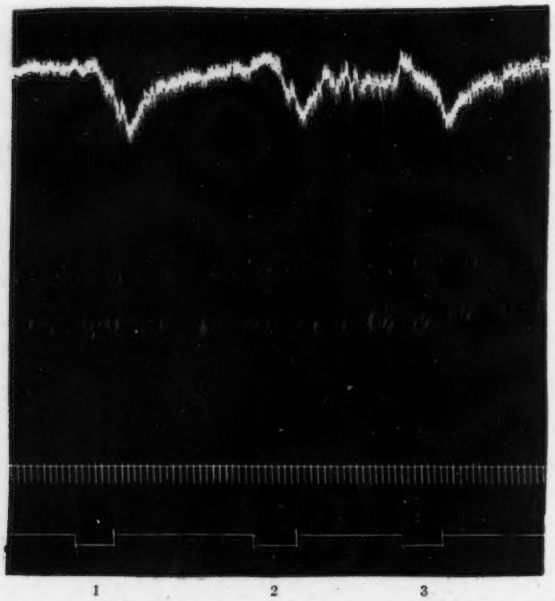

Fig. 4. Dog. Thorax opened. Femoral nerve. Strength of stimulus in Z units, *1*, 485; *2*, 2425; *3*, 4850.

SUMMARY

In our experiments, respiration affected in no manner whatever the production of the fall in blood pressure upon central afferent nerve stimulation. Opening the thorax had no effect upon the production of the depressor response from the vasomotor mechanism.

With a rate of 7 interruptions per second we found it extremely difficult to bring about pressor responses but depressor responses were readily elicited regardless of the condition of the thorax.

The average thresholds for the depressor response in dogs, 8.3 with thorax closed and 7.3 with thorax opened, are about the same as that found by Martin and Lacey upon decerebrate cats, 8.5 Z units.

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THE ACTION OF THE AUTONOMIC DRUGS ON THE SURVIVING STOMACH

A Study on the Innervation of the Stomach

MAURICE I. SMITH

From the Pharmacological Laboratory of the University of Nebraska Medical School, Omaha[1]

Received for publication April 9, 1918

A study of the literature dealing with the innervation of the stomach and the effects of drugs upon it discloses that all such investigations were carried out on the whole organ, whether in situ or isolated. There does not seem to have been any consideration given to the possibility that the different parts of the viscus may have different innervations and that the effect of a given drug on one region of the stomach may not necessarily be the same as its effect on another portion of this organ.

That the innervation of the stomach may not be uniform at all points and in all animals, at least in as far as the sympathetic nerve supply of it is concerned, might be surmised from the fact that different observers have reported varying effects on the movements of this organ following the stimulation of the splanchnic nerves. Schiff (1), Morat (2), Dixon (3) and others have shown the splanchnics to supply motor fibers to the stomach, while Wertheimer (4) and Elliott (5) may be mentioned among those observers who found the stomach movements to be inhibited from the stimulation of the splanchnics. Openchowski (6) demonstrated both motor and inhibitory effects on the stomach of the dog and the rabbit following stimulation of the splanchnics, while May (7) on the other hand, stimulating the same nerves in the cat, dog, rabbit and monkey observed no appreciable effects on its movements.

As to the effects of drugs on the movements of the stomach, the work of Schutz (8) may be mentioned, who limited his observations to the action of a series of drugs on the whole isolated stomach of the

[1] Some of the earlier experiments of this research were carried out in the pharmacological laboratory of the University of Michigan.

dog. Later Glaessner (9) made similar observations on the whole excised stomach of the frog.

The action of suprarenal extract on the movements of the stomach undoubtedly received the greatest attention in the hands of various observers but no uniform results have been reported. Boruttau (10) working with rings of the frog's stomach found that suprarenal extract caused a relaxation of tonus. Elliott (5) observed similar effects from the extract on the cat's stomach, as did Langley (11) in the case of the stomach of the cat and the rabbit. Dixon (3) however found suprarenal extract to cause tonic contraction and augmented automatic movements of the frog's stomach.

METHOD AND SCOPE

The present work was carried out on strips of the surviving stomach suspended in oxygenated Tyrode's solution kept at constant body temperature. The movements of the strips were recorded on a slowly revolving kymograph by means of a light heart lever sufficiently weighted. The drugs were added in definite amounts to the Tyrode solution containing the suspended strip. Just as soon as the effects of a drug were noted the solution was drawn off and fresh Tyrode's solution, warmed to body temperature, was replaced. A period of at least five minutes and usually a longer interval of time elapsed before a second trial with the same or another drug was made.

The strips were generally taken from the following regions of the stomach: (1) antrum; (2) preantrum,—well defined in the rabbit's stomach, less so in that of the cat and dog; (3) body of the stomach, the portion extending from the preantrum to a point opposite the oesophageal insertion; (4) fundus, or the rounded cul-de-sac to the left of the oesophageal orifice.

In most cases longitudinal strips were used though at times strips corresponding to the circular as well as the oblique fibers of the stomach wall were employed. No material difference could be observed in the behavior of the longitudinal, circular or oblique strips of a given region toward the drugs used.

Both surfaces of the stomach, the anterior as well as the posterior, were examined. In a few instances strips from the lesser and greater curvatures were employed and several experiments were made with the pyloric and cardiac sphincters. At least two experiments were carried out for each region of either surface. As a rule fresh tissue was used

but in a few experiments tissue that was kept in the cold for about twenty-four hours was used, which was found to respond perfectly well. Strips from the stomach of the guinea pig, rabbit, cat, dog and the human subject[2] were utilized in this work.

The observations were limited to the effects of those drugs that act on the autonomic nervous system in the hope that this might throw some light on the innervation of the several regions of the stomach. Epinephrin and nicotine were used as acting on the sympathetic structures, "receptive substance" and ganglia respectively, and pilocarpine and atropine, acting presumably on the parasympathetic endings and antagonistic to each other. Barium chloride was frequently employed to test the irritability of the tissue. Thus if a strip failed to respond to

Fig. 1. Body of rabbit's stomach, anterior surface. Contraction and increased tonus from 1 mgm. pilocarpine hydrochloride. Relaxation from 0.1 mgm. atropine sulphate. (In this as in subsequent tracings contraction is indicated by downward movement of lever.)

barium it was not considered suitable for this work, and it was discarded. It may be remarked however that often stomach strips failing to respond to barium chloride responded perfectly well to pilocarpine.

RESULTS

Effects of pilocarpine and atropine. Pilocarpine and atropine produced a uniform effect upon all the regions of the stomach, and in all

[2] Owing to the kindness of the medical staff of the University Hospital it was possible for me to secure the stomach of a patient who died about three hours previously of ruptured aneurysm.

the species of animals studied. Pilocarpine uniformly produced a contraction in dilutions of from 1: 10,000 to 1: 100,000, while atropine antagonized the effects of pilocarpine in solutions one-tenth as strong, and produced a relaxation of the tissues. One typical illustration of the effects of pilocarpine and atropine is shown in figure 1. On adding 1 mgm. of pilocarpine hydrochloride to about 100 cc. Tyrode's solution in which a strip from the anterior surface of the body of the rabbit's stomach was suspended a marked contraction resulted. This was promptly counteracted, and relaxation occurred, upon the addition of 0.1 mgm. atropine sulphate. Similar effects were noted from these drugs on the pyloric and cardiac sphincters.

Effects of nicotine. A solution of the hydrochloride of nicotine was used. The effects of this drug were noted in dilutions of from 1: 10,000 to 1: 1,000,000 upon all regions of the stomach, including the sphincters, of all the animals studied. A tonic contraction such as is shown in figure 2, produced by 3 mgm. nicotine hydrochloride added to about 100 cc. Tyrode's solution in which a strip from the anterior surface of the human antrum was suspended, is the rule.[3] The contraction usually appeared promptly and soon passed off, so that the strip returned to its original condition, or it passed into a state of relaxation. The shortness in duration of the nicotine contraction in the above mentioned concentrations does not seem to be due to a paralysis of any local nerve mechanism for the addition of another dose of the drug to the same solution often produced a similar contraction, and repeated contractions were obtained from successive doses of the

Fig. 2. Human stomach. Antrum, anterior surface. Shows tonic contraction from 3 mgm. nicotine hydrochloride.

[3] In a number of experiments no effect could be obtained from nicotine, even though the strips contracted spontaneously and responded well to the other drugs employed.

drug when added to fresh Tyrode's solution at short intervals of time.

Occasionally the contraction produced by nicotine was preceded by a slight relaxation. This was noted in a few instances in the case of the antrum, body and fundus of the rabbit's stomach. The cardiac sphincter of the cat's stomach relaxed from nicotine, while the fundus relaxed in one experiment and contracted in two.

TABLE 1

Effect of epinephrin on the different regions of the stomach

STRIP	GUINEA PIG	RABBIT	CAT	DOG	HUMAN
Pyloric sphincter..		Contraction	Contraction		Contraction
Antrum.....		Relaxation	Relaxation		Relaxation
Preantrum..		Relaxation	Relaxation	Relaxation	
Body.....	Anterior surface and greater curvature relaxation; posterior surface and lesser curvature contraction	Contraction*	Relaxation	Contraction	Relaxation
Fundus.....		Contraction	Relaxation	Contraction	
Cardiac sphincter..			Contraction	Contraction	

* Deviations from this are discussed in the text.

Effects of epinephrin. The hydrochloride of epinephrin[4] was used in dilutions varying from 1:100,000 to 1:10,000,000. The results of this drug are best presented in table form, from which it will appear that the response of the various strips to it is not the same for all regions of the stomach, nor do the strips of the same region of different animals respond alike.[5]

[4] Made from adrenalin of Parke, Davis and Company.

[5] It may be recalled that small doses of epinephrin may produce the opposite effect of a larger dose. This was constantly borne in mind and for each result in the accompanying table several trials were made with varying doses.

The body of the stomach of the guinea pig, the only portion of this animal's stomach definitely worked out, presented a different reaction to epinephrin for its two surfaces and curvatures. Thus while the anterior surface and greater curvature relaxed, the posterior surface and lesser curvature strongly contracted.

In the other animals in which a complete record was obtained for all the regions of the stomach, considerable differences also prevailed. All the regions of the cat's stomach, with the exception of the sphincters, relax from epinephrin. The stomach of the rabbit, however, as well as that of the dog contracts in the main from epinephrin, although some regions relax. Thus, the antrum and the preantrum are the only parts of the rabbit's stomach that definitely and constantly relax. In one experiment the posterior surface of the body of the rabbit's stomach was noted to slightly relax before contracting (fig. 3). The region of the dog's stomach that uniformly relaxed from epinephrin is the preantrum. Strips from the antrum repeatedly failed to respond in any manner even though they were beating spontaneously and responded to barium chloride, pilocarpine, atropine and nicotine with great avidity. The body and the fundus of the dog's stomach were found to uniformly contract.

Fig. 3. Body of rabbit's stomach, posterior surface. Strong contraction preceded by slight relaxation, produced by 0.1 mgm. epinephrin hydrochloride.

That the reaction of the larger part of the rabbit's and the dog's stomach to epinephrin by a contraction is not merely a peculiarity of surviving tissue but that the same reaction prevails in the living animal was verified by two experiments on the rabbit and one on the dog in which the movements of the stomach in situ and the reaction to epinephrin were observed.

The animals were anesthetized, submerged in a bath of normal saline

at 38°C., the stomach exposed by a median incision of the abdomen and the Cushny myocardiograph tied into the anterior surface of the body of the stomach to record the movements of a strip of about 4 cm.

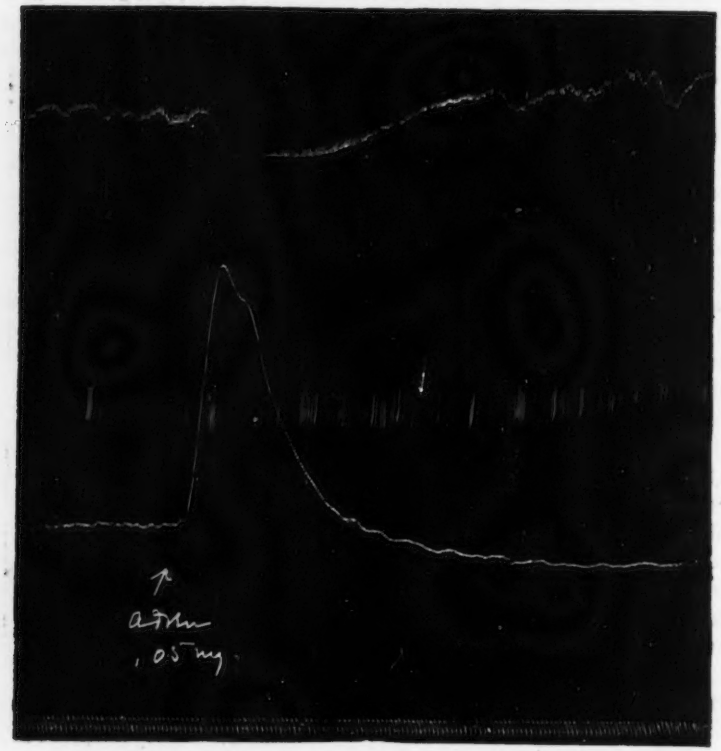


Fig. 4. Effect of epinephrin on the movements of the body of the rabbit's stomach in situ. Upper tracing, movements of stomach; middle tracing, blood pressure; lower tracing, time in seconds. Note the contraction of this portion of the stomach following the rise in blood pressure.

thereof. The blood pressure was recorded at the same time.[6] Following the injection of a small dose of epinephrin into the jugular vein there was the usual rise in blood pressure, and a well marked contrac-

[6] In the dog the vagi were divided and artificial respiration administered.

tion of the portion of the stomach recorded, which lasted about the same length of time as the rise in blood pressure (fig. 4).

The effect of epinephrin on strips of the human stomach was that of relaxation in as far as it was possible to ascertain by the available material. Both surfaces of the antrum and the body of the human stomach were found to relax from this drug.

The effects of epinephrin on the sphincters were obtained with difficulty. The excised muscle of the sphincter is strongly contracted and thus responds slightly if at all to augmentory drugs. The pyloric sphincter of the stomach of the cat, rabbit and the human subject was found to contract slightly in a few instances while in most cases no reaction could be obtained. The cardiac sphincter of the cat's stomach contracted well from epinephrin and that of the dog's stomach but slightly. The cardiac sphincter of the rabbit's stomach failed to respond to this drug. It would seem that when a reaction to epinephrin is obtainable with the sphincters, it is a contraction, which is in agreement with the observations of Elliott on the effects of epinephrin on the pyloric sphincter of the cat (5). Langley (11) however has shown that suprarenal extract (1 cc. "tabloid 1 in 6") produced a relaxation of the cardiac sphincter of the rabbit. I am unable to corroborate this observation by the method I have used, and I can not account for the disparity of results.

DISCUSSION

The interpretation of the results obtained with pilocarpine and atropine upon the stomach is rather dubious. The action of these drugs, it is generally held, is upon the parasympathetic nerve endings and it was hoped that the reaction of strips from different regions of the stomach to them might show the distribution of the vagus nerve to this organ. The uniform response of the various strips to pilocarpine by a contraction, and to atropine by a relaxation cannot be taken however as evidence that all the regions of the stomach are supplied by motor fibers from the vagus, since it has been shown by Openchowski (6), Langley (12), May (7), Elliott (5) and others that stimulation of the vagus relaxes at least some parts of the stomach, especially the region of the cardia. It must be remarked however that the relaxation of part or all of the stomach from vagus stimulation as reported in the literature is a primary effect and that the secondary effect, it is generally agreed, is a powerful contraction of the whole organ. It

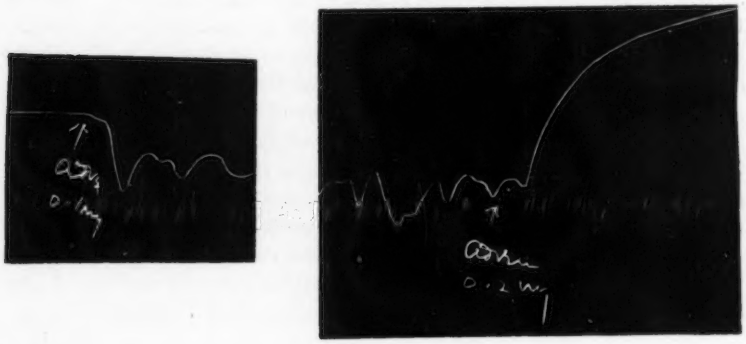
may well be that the primary relaxing effect upon some parts of the stomach resulting from stimulation of the vagus cannot be demonstrated on excised tissue. This is made more probable by the observation of Langley (12) that frequently the primary as well as the secondary effect from vagus stimulation is a contraction, and that in the exposed stomach the vagus inhibitory effects are less marked and often inconstant. The other alternative is that the contraction produced on all the parts of the stomach by pilocarpine is due to some mechanism other than through the vagus.

No very clear explanation can be set forth for the effects of nicotine upon the different regions of the stomach. It was found, as pointed out earlier, that as a rule, whenever effective, nicotine produces a contraction. In about one-third of the number of experiments, however, (about twenty out of fifty-five), the tissues although beating spontaneously and reacting well to the other drugs, failed to react to nicotine. Is this motor effect of nicotine when elicited to be ascribed to its action on ganglia, and is a positive effect on a given strip to mean the presence of ganglia, while a negative result is to indicate absence of ganglia in that particular strip? Such an explanation is plausible although it can not be stated with any degree of definiteness. While there are ganglionated structures scattered all through the walls of the stomach (plexuses of Auerbach and Meissner), special groups have been shown to occur in greatest abundance in the pyloric and cardiac regions (Openchowski (6), Keith (13)). An analysis of my experiments does not show strips from these regions to respond to nicotine more frequently than strips from other regions. Indeed, the greatest number of failures to respond to this drug occurred among the strips taken from the antrum, while those from the preantrum responded in all cases. Strips from the fundus (region of the cardia) compare with those taken from the body of the stomach as regards their reaction to nicotine.

The significance of the reaction of the different regions of the stomach to epinephrin is clear. Lewandowski (14), Langley (11), Elliott (5) and others have shown that the effect of epinephrin on a tissue corresponds to the stimulation of the sympathetic innervation thereof and that the action of the drug is augmentory or inhibitory depending upon as to whether the corresponding sympathetic nerve supply is motor or inhibitory. The different reactions of the various strips to epinephrin clearly show that the sympathetic innervation of the stomach is not the same in all animals and is different for different regions

of the stomach in the same animal. It appears that of the usual laboratory animals the cat alone has an inhibitory sympathetic nerve supply of the whole stomach, with the exception of the sphincters. This confirms the observations of Elliott (5) who found by recording the volume changes of the cat's stomach a complete relaxation of the whole organ upon the administration of epinephrin as well as upon the stimulation of the splanchnics.

The sympathetic innervation of the rabbit's and dog's stomach on the other hand, is augmentory in the main, except that of the antrum and preantrum of the former and the preantrum of the latter



a b

Fig. 5. Body of dog's stomach. Posterior surface. Effect of epinephrin before and after ergotoxine. *a*, 0.1 mgm. epinephrin causes a contraction. *b*, 0.2 mgm. epinephrin, after 2 mgm. ergotoxine, produces a relaxation.

animal, since only the antrum and preantrum of the rabbit and the preantrum of the dog relax from epinephrin, while the other regions contract.

That the parts of the dog's stomach (and probably of the rabbit's as well) contracting from epinephrin are not devoid of sympathetic inhibitory fibers, was shown by the application to the stomach of Dale's epinephrin vasomotor reversal produced by ergotoxine (15). A strip from the posterior surface of the body of the dog's stomach was suspended in the usual manner in Tyrode's solution and treated with a small dose of epinephrin. The usual contraction followed. The solution was withdrawn, fresh Tyrode's solution replaced and about 2 mgm. ergotoxine were added to this. After a few minutes the reac-

tion of the strip to epinephrin was again tested. Instead of the usual contraction a very marked relaxation occurred (fig. 5). A similar reversal was obtained in another experiment on the fundus of the dog's stomach This proves in another way the earlier observation of Openchowski (6) that the sympathetic nerve supply of the dog's stomach is both motor and inhibitory.

The sympathetic innervation of the human stomach, as can be inferred from the reaction of the strips to epinephrin, is inhibitory, except of course the sphincters.

It seems contrary to expectations that the innervation of the dog's stomach is more like that of the rabbit's than that of the cat's. Besides the dog being a carnivorous animal like the cat and unlike the rabbit, it is a matter of common observation that anatomically the dog's stomach conforms more closely to that of the cat than to that of the rabbit. Physiologically too, in the matter of absorption, and the ease with which the dog can be induced to vomit would put his stomach nearer that of the human and the cat than that of the rabbit. It is perhaps justifiable to conclude that the sympathetic innervation of the stomach has nothing to do with the process of vomiting since the innervation of the stomach of the dog that can be induced to vomit very readily is very much like the sympathetic innervation of the stomach of the rabbit, that is entirely incapable of vomiting.

SUMMARY AND CONCLUSIONS

1. Pilocarpine causes a contraction of all regions of the surviving stomach of the guinea pig, rabbit, cat, dog and the human subject. Atropine antagonizes the action of pilocarpine and produces a relaxation.

2. Nicotine likewise produces a contraction of all parts of the stomach of the animals enumerated, except some parts of the cat's stomach (fundus, cardiac sphincter) which may relax, and some parts of the rabbit's stomach (antrum and body) which may slightly relax before contracting.

3. The reaction of the different parts of the stomach to epinephrin may be that of relaxation (cat and human), or relaxation of some parts and contraction of others (guinea pig, rabbit, dog). The reaction of the sphincters to epinephrin is always that of contraction.

4. Those regions of the dog's stomach that contract from epinephrin can be made to relax therefrom after ergotoxine, showing that there is

an inhibitory sympathetic innervation to those parts as well as an augmentory, although the latter predominates normally.

5. It is concluded from this that the sympathetic innervation of the stomach is inhibitory in some animals (cat and the human), while in other animals (guinea pig, rabbit and dog) it is inhibitory for certain regions and predominantly augmentory for others.

The sympathetic innervation of the sphincters of the stomach appears to be augmentory.

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EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

I. Normal Temperature Variations and the Temperature Effects of Operative Procedures

LILLIAN M. MOORE

From the Rudolph Spreckels Physiological Laboratory of the University of California

Received for publication April 15, 1918

INTRODUCTION

This paper is the first of a series of experimental studies on the various physiological and anatomical factors which tend to bring about the relatively constant temperature found in birds and mammals.

It is commonly agreed that two fundamental processes are involved in the regulation of temperature: heat production and heat dissipation. Anything which increases destructive metabolism increases heat production and tends to bring about a corresponding rise in body temperature. If, on the other hand, the heat production remains constant, the temperature varies according to the amount of heat given off or retained. It is recognized that loss of heat occurs through radiation and conduction and through evaporation of water. The loss through radiation and conduction is affected by vasomotor changes; that by evaporation is affected by vasomotor changes and by the rate of activity of the sweat glands and by the rate and depth of the breathing movements. Body temperature must be thought of, therefore, as a balance between heat production and heat dissipation, the balance remaining fairly constant in all warm-blooded animals.

How is the balance maintained? Many workers have assumed the existence in the brain of a convenient, hypothetical "heat center," which acts as a thermo-regulator, automatically altering the heat production or dissipation so as to maintain the balance in ordinary conditions. If the center is stimulated, a higher temperature results; if it is depressed or destroyed, the temperature falls. These writers have

differed among themselves as to the location, number and exact function of the centers assumed.

In contrast to these views, others think of the heat regulation as analogous to the regulation of blood pressure. The control of blood pressure is never ascribed to a specific center but is regarded rather as the mean result of various physico-chemical factors. There is an even greater constancy in the acidity and calcium content of the blood, which is accounted for by physical and chemical equilibria without the intervention of special "centers." A similar balance between heat production and heat loss might well result in a constant body temperature. It does not seem to have been thought necessary to assume "centers" for the control of the osmotic pressure of the blood nor of the number of red corpuscles to the cubic millimeter.

As Henderson (1) says:

> Further research reveals similar equilibria concerning carbon, sulphur, phosphorus and other elements . . . . water, salt, sodium bicarbonates, glucose and the like. It is perceived that the equilibria of *temperature*, of volume, of alkalinity, which involve physico-chemical states are truly analogous phenomena.

Since the available experimental evidence for a specific temperature regulatory mechanism, though profuse, is conflicting and often unconvincing, it seemed worth while to investigate further the factors concerned. Before beginning the experiments necessary for this purpose, it was imperative to determine, first, the range of normal temperature of the animals used; and second, the variable conditions which might exert an effect on their temperature and thus prove a source of error in the experimental results. With this object in view the observations recorded below were obtained. Rabbits were employed as the experimental animals.

The author gratefully acknowledges the guidance of Prof. S. S. Maxwell in this investigation.

I. THE NORMAL TEMPERATURE OF THE RABBIT

It is impossible to establish a temperature norm for the rabbit because of its extreme variability in this animal. The range however, has rather definite limits. Pembrey (2) gives extremes of 37° and 40.8°C.; Simpson and Galbraith (3) 39° and 40°C.; Krehl (4) 38.3° and 39.9°C.; Freund (5) 38.6° and 39.6°C.; Davidson and Friedman (6) 38.5° and 40°C.; Bock (7) 38.6° and 40.9°C.; Burnett (8) 38.6° to 40°C.; Hale

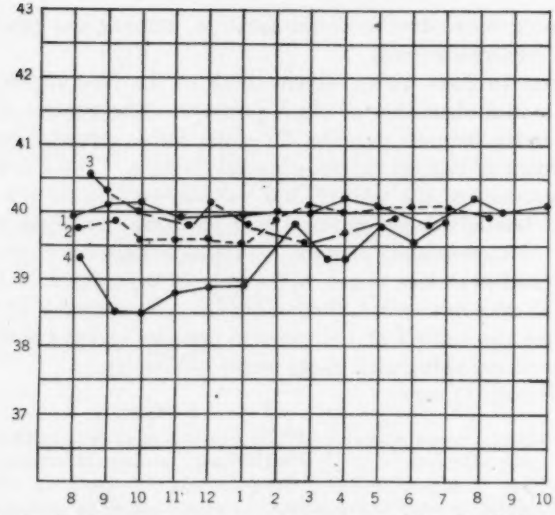

Chart 1. Daily temperature variations in rabbit 7. Ordinates, degrees Centigrade; abscissae time in hours. *1*, November 17; *2*, November 13; *3*, November 7; *4*, November 24.

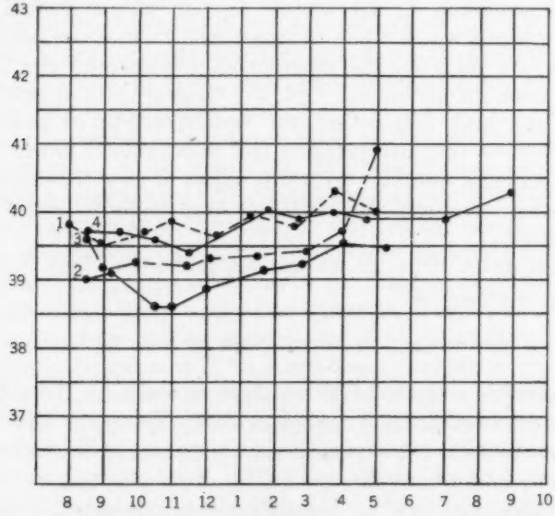

Chart 2. Daily temperature variations in rabbit 10. Ordinates, degrees Centigrade; abscissae time in hours. *1*, December 5; *2*, December 6; *3*, December 7; *4*, December 14.

White (9) 37.3° and 39.9°C. Frothingham and Minot (10) decide from a series of two readings daily that although these and other observers find variations from 2° to 4°C. a constant range of 2°C. would be utilizable in drawing valid conclusions in experimental work. The data given in this article were obtained from observations on 22 rabbits. The observations extended over a period of eighty-six days and included 774 readings.

Method. The rabbits were kept in large boxes in the experimental room and were never moved except to make the observations. They were then handled in such a way as to cause as little excitement as possible, struggling seldom occurring. They were fed on barley and hay daily about 6 p.m. The temperature was taken per rectum by standardized clinical thermometers inserted to a depth of two or more inches and left in for two minutes. The temperatures were recorded hourly from 8 a.m. to 6 p.m. and in many cases to 9 or 10 p.m. The observations of eleven rabbits were made on from two to thirteen successive days, the animals being kept as nearly normal as possible in the meantime. In the remaining cases the readings were taken during a single day for each rabbit.

Charts 1 and 2 are specimen curves of the daily temperature variations of two rabbits. In some cases the range was much greater, one varying from 38.4° to 41.2°C. in the course of one day. The extreme of all observations were 38.2° and 41.4°C. The mean was 39.68°C. The variability range within which two-thirds of the normal readings should fall was calculated by statistical methods and found to be 39.4° and 39.9°C.

These figures and curves show that one temperature observation cannot be used as the norm for that rabbit. A change above or below this one reading cannot be considered to be experimentally produced unless it is great enough to fall beyond the range of normal variability. Some workers consider a steady rise or fall as experimental compared with the fluctuating normal. It is conceivable that the range would need to be determined for any given experimental environment. The extremes obtained in this series were in all cases higher than those reported by others. In no case was a normal temperature below 38°C. observed, although other investigators frequently report a minimum between 37° and 38°C. It might be well to state that the majority of my experiments were performed in California during the months from August to May. A smaller number were carried on in Idaho in the months of June and July. There was, however, no noticeable differ-

ence in the average range of temperature in the two series. In neither case, however, was the external temperature extremely low.

II. EFFECT OF EXERCISE, FOOD, SEX, POSTURE, ON THE TEMPERATURE OF THE RABBIT

A. Exercise. Kraus (11) reports that a rabbit in a treadmill showed a rise from 39.05° to 40.1°C. during seven minutes' work, the temperature returning to normal in thirty minutes. This accords with the

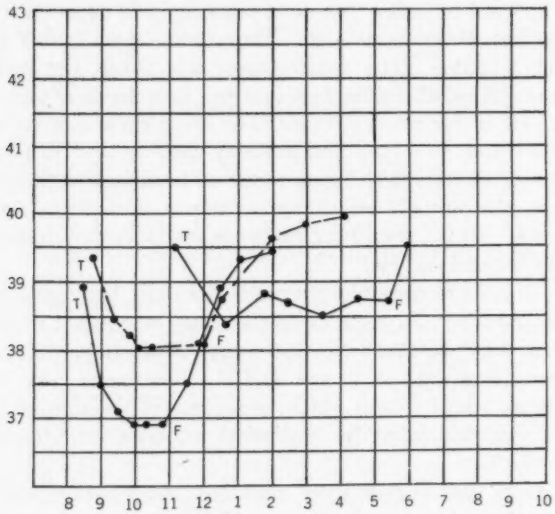

Chart 3. Temperature variations in three rabbits fastened to operating boards. Ordinates, degrees Centigrade; abscissae time in hours. *T*, tied; *F*, free.

well known effects of exercise on the temperature of man and other animals. No exact observations were made in this series; care was taken, however, in every experimental case to prevent unusual muscular movements.

B. Food. The rise in temperature after the taking of food is also familiar. After feeding, the rabbits used in these experiments showed a rise of 0.5°C. or more. Inanition results in a corresponding fall. The temperature of one rabbit varied from 39.5° to 32°C. during two weeks' starvation. All the animals used in this series were fed regu-

larly and never prior to nor during an experiment, thus eliminating any possible marked deviation due to feeding.

C. Sex. It has been reported that the temperature of females often ranges slightly above that of the males. The observations on normal rabbits cited above tend to confirm this.

D. Posture. It is well known that the temperature of rabbits falls when they are tied down. Kraus (11) noted a fall of 0.2° to 0.4°C. in five to ten minutes. Chart 3 gives the temperature changes which I

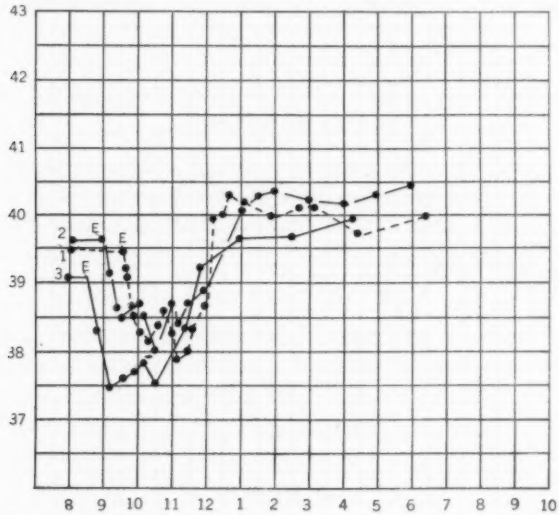

Chart 4. Temperature variations due to ether anaesthesia. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 6, ether administered for twenty minutes; *2*, rabbit 5, ether administered for twenty minutes; *3*, rabbit 4, ether administered for five minutes. *E*, Ether.

found in rabbits fastened to an operating board, back uppermost, for varying length of time. The eight cases recorded gave a fall of 1° to 2°C. in one and one-half to two hours. After reaching the minimum, the temperature remained practically stationary as long as the rabbits were kept in that position, except that occasionally struggling caused a rise of 0.2° to 0.3°C.

It would seem advisable in cases in which it is necessary to keep a rabbit extended for any length of time, to obtain this minimum temperature before attempting to secure experimental changes. After

releasing the rabbits, the temperature rose generally 0.1 to 0.5°C. higher than at the beginning of the experiment. The extremes were 36.95° and 39.7°C.

III. EFFECTS OF ANAESTHETICS ON THE TEMPERATURE OF THE RABBIT

Anaesthetics in general are reported as causing a lowering of body temperature during and following their administration. Ether, according to Angelesco (12) causes a fall until the animal "comes out,"

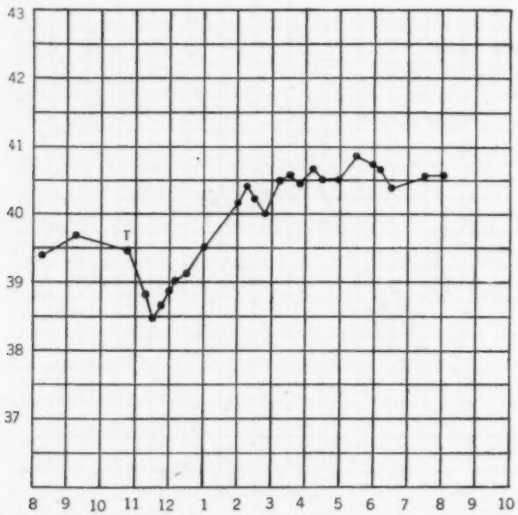

Chart 5. Temperature variations following trephining of the skull under nitrous oxide anaesthesia. Ordinates, degrees Centigrade; abscissae, time in hours. *T*, trephine.

and then a rise. The fall is due to vasodilatation and lessened muscular activity and tonus. Hale White (9) as preliminary to experiments on the "heat centers" tested the effect of ether and concluded that it did not cause an abnormal temperature. Most workers, however, agree that there is a fall in ether anaesthesia amounting often to as much as 5°C.

Inconsistencies in the results obtained in experimental work in this laboratory in relation to temperature led to a reinvestigation of the effects of ether and nitrous oxide on body temperature. Chart 4 gives

specimen curves of the temperature variations due to ether anaesthesia. It will be noted that there is a fall of 1.5°C. in the first hour after the cessation of etherization, followed by a rise of 1°C. above the initial temperature in the subsequent two hours. The extremes were 37.4° and 40.9°C. Most writers fail to report the final rise. Nitrous oxide gas gave similar changes but within narrower limits. In ten other observations on the effects of ether and of nitrous oxide on temperature and in some two hundred operative experiments on rabbits in which ether or nitrous oxide was used as an anaesthetic similar changes were noted.

IV. THE EFFECT OF OPERATIVE PROCEDURE ON THE TEMPERATURE OF THE RABBIT

Hale White (9) reports dummy experiments in which trephine openings were made in the skull. In some cases the white matter was injured. He finds abnormal temperatures in only a few cases. His readings, however, were taken at intervals of several hours so that if high temperatures occurred he might easily have failed to observe them.

In three rabbits in this series trephine openings were made in the skull under ether or nitrous oxide anaesthesia. They all showed an initial fall of 1° to 2°C. followed by a rise of more than 1°C. above normal. The extremes were 36.9° and 40.9°C. Chart 5 gives a specimen curve of the above.

SUMMARY

1. The normal range of variability in the temperature of the rabbits used, including daily variations, was found to be between 39.4° and 39.9°C. with an average of 39.68°C. and extremes of 38.2° and 41.4°C.

2. Anaesthetics necessary in operative procedure cause a marked variation in the temperature of the rabbit; ether an average fall of 1.4°C. followed by an average rise of 1°C. above the initial temperature; nitrous oxide gas a similar but less marked change. The extremes were 37.4° and 40.9°C.

3. The temperature of rabbits which are tied down falls 1° to 2°C. in one to two hours and remains stationary until they are released. It then rapidly rises to 0.5°C. above the initial temperature. The extremes were 36.95° and 39.7°C.

4. Operative procedure such as trephining the skull causes an aver-

age fall of 1.9°C. followed by a rise of 1°C. or more above the initial temperature. The extremes were 36.9° and 40.8°C.

CONCLUSION

Hyperthermia in rabbits cannot be considered to be experimentally produced unless it exceeds the normal and operative variations.

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EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

II. Relation of the Corpus Striatum to the Regulation of Body Temperature

LILLIAN M. MOORE

From the Rudolph Spreckels Physiological Laboratory of the University of California

Received for publication April 15, 1918

HISTORICAL STATEMENT

A rise in temperature following injury to various parts of the brain tissue of the rabbit was long ago reported by Tscheschichin (1), Schrieber (2), Bruck and Günther (3), Eulenberg and Landois (4) and others. The rise was generally accompanied by muscular spasms. Aronsohn and Sachs (5) in 1885 first described "heat puncture" caused by puncturing or otherwise injuring or stimulating the medial side of the corpus striatum. They reported a rise of 1.7 to 2.4°C. lasting several days. These observations led to the theory that there exists in the brain of birds and mammals a special "heat center" which automatically regulates the heat production and dissipation in such a way as to maintain a constant body temperature.

Subsequent investigators affirm the existence of various other "heat centers;" Ott (6) locates the "center" in the corpus striatum, optic thalamus, tuber cinereum and pons; Girard (7) in the corpus striatum, optic thalamus, septum pellucidum and corpus callosum; Steerath (8) in the optic thalamus; Aisenstat (9), Gottlieb (10), Ito (11), Babinsky and Lehmann (12) and Nikolaides and Dontas (13) in the corpus striatum; Hale White (14) in the corpus striatum and optic thalamus; Jacobj and Roemer (15) in the lateral ventricles; Isenschmidt and Krehl (16) in the tuber cinereum; Citron and Leschke (17) somewhere above the corpora quadrigemina.

Recent work by Barbour and Wing (18), Demming (19), Prince (20) and Hashimoto (21) in Hans Meyer's laboratory in Vienna, consists in the application of pyretics and antipyretics to the "heat centers,"

which they find to be in the mid-ventral part of the caudate nucleus of the corpus striatum. This center they consider to be dual in nature, that is, made up of a thermogenic or heat center, and a thermolytic or cold center. Pyretics stimulate the former and depress the latter; antipyretics have a reverse action. They say, incidentally, that in some cases of puncture a fall instead of a rise in temperature was obtained. Their work is confirmed by Cloetta and Waser (22). A number of other investigators locate a special temperature regulatory "center" somewhere in the brain.

The above evidence would seem to indicate that a special "heat center" does exist in the brain. The results and conclusions are, however, in many cases open to criticism.

"Puncture fever" as described by Bruck and Günther (3), by Schrieber (2) and others was accompanied by muscular spasms, the heat production from which would need to be considered before the rise in temperature could be said to be produced by injury to a heat center.

Aronsohn and Sachs (5) did not obtain uniformly high temperatures but in many cases reported a rise of less than 1°C. (0.2 to 1°), nor were their lesions always in the corpus striatum. The medial edges of the hemispheres, the septum pellucidum and the lateral ventricles were often the seat of injury. The great length of time before the rise was obtained admits of the possibility of infection as the exciting cause. White's experiments on the optic thalamus are less convincing. When carefully analyzed, the latter's results seem to serve as negative evidence for a specific "heat center" in the corpus striatum or optic thalamus.

Barbour and Wing (18), Prince (20), Hashimoto (21) and other workers in Hans Meyer's laboratory obtained a rise in temperature only when the mid-ventral portion of the caudate nucleus of the corpus striatum was injured. In one series of twenty-five experiments, four cases showed a temperature of 41°C. or above. These were all through the mid-ventral portion of the caudate nucleus. Seven other punctures in the same limited region ranged from 39.4 to 40.9°C. which cannot be considered experimental hyperthermia. All the other punctures were through the anterior or posterior part of the caudate nucleus, and resulted in temperatures from 39.7°C. to subnormal. This evidence again tends to deny the presence of a specific heat center.

Similar criticism may be made of the results and conclusions of other investigators who describe "heat punctures."

Pembrey (23) opposed the "heat center" theory of temperature regu-

lation, considering the centers as purely hypothetical. The compensation between heat production and heat loss brought about by physical and chemical means was, according to him, sufficient to regulate temperature (24). He found that young mammals and birds born in a well developed condition such as the guinea pig and chick, were fully active and produced enough heat to maintain a constant temperature; while helpless newly born animals such as mice and pigeons were able to regulate their temperature only to a moderate degree. In cold they could not respond by increased activity until ten or fifteen days old, at which time muscular activity came on and increased vasomotor tonus was evident. Then the temperature was regulated by heat production and heat loss with no apparent need of a heat "center."

Pembrey (25) also found that hibernating animals could awaken with an increase of body temperature after the corpus striatum had been removed. Pembrey (26) showed that the awakening of a dormouse is accompanied by violent shivering, the temperature often rising 10° to 20°C. in a few hours. Calorimetric measurements by Pembrey (27) show that the heat produced is sufficient to account for the rise without the intervenion of a heat center. Du Bois (28) showed that hibernating animals with motor paralysis had only a small rise in temperature on awakening.

Pembrey and Mutch (29) also showed that tetrahydro-β naphthylamine caused a marked rise in temperature only when violent muscular activity and convulsions resulted. If chloroform were given during the rise to prevent muscular movements the temperature fell until the effect of the chloroform had worn off. Tetrahydro-β naphthylamine also did not cause a rise in rabbits if the muscles were paralyzed by cutting the motor nerves or by curare.

Fredericq (30) found that removal of the cerebral hemispheres in pigeons produced no variations in the daily temperature curve. Corin and Van Beneden (31) obtained similar results and observed no change in the CO_2 exchange of such pigeons. Goltz's (32) well known decerebrate dog had a temperature only slightly below normal.

Du Bois (33) found that the corpus striatum, midbrain and cerebrum of marmots, pigeons and rabbits could be destroyed thus eliminating all the hypothetical heat centers without loss of temperature regulation. Mosso (34) also denies the existence of heat centers affirming that hemorrhage and excitement are the cause of the rise of temperature following punctures. He obtained a rise by the injection of cocaine after the "heat centers" were removed.

Wilson (35) from a comprehensive neurological study of the corpus striatum concludes that it cannot be termed a heat center. Injury to the corpus striatum causes hypertonicity of the muscles, often resulting in tremors sufficient to cause a marked increase in the heat production. This together with the vasoconstriction due to the increased tonus in the walls of the blood vessels could produce hyperthermia.

Sachs and Green (36) in a recent publication do not confirm the heat center theory. Lesions or stimulation of the caudate nucleus in rabbits and cats gave no greater rise than controls. Hill (37) also refutes the claim of a special heat center.

The evidence just cited points to the probability that temperature regulation is controlled entirely by factors which are not dependent on specific "heat centers" in the brain. Still we cannot as yet say why most warm-blooded animals maintain a higher level of body temperatire than the majority of cold-blooded animals. The "heat center" theory, however, conveniently accounts for this and is, therefore, still accepted by many physiologists.

The experiments reported in this paper on the relation of the corpus striatum to the regulation of body temperature were begun with the idea of applying drugs to the "heat centers" after the method of Barbour and Wing (18). The difficulty met with in locating a definite center and the variability in the results obtained by puncture, however, threw so much doubt upon the existence of such centers that the attempt was made to reinvestigate the whole matter.

EXPERIMENTS ON THE "HEAT CENTER"

A. Puncture. Since the caudate nucleus of the corpus striatum is generally accepted as the most probable "heat center," it is the only one considered in this part of my work.

The punctures were made according to the methods of Aronsohn and Sachs (5). The hair on the head of the rabbit was removed, a longitudinal incision made in the skin and a trephine opening 1 cm. in diameter made 1 mm. to the right of the longitudinal suture and 3 mm. anterior to the coronal suture. Into this opening was screwed a metal cylinder with a small central hole through which the puncture needle 1 mm. in diameter was inserted to varying depths. The needle could be removed or left in place after the puncture was made.

Aseptic precautions were used throughout the operation and the

wound was covered with a sterile cotton cap held in place by flexible collodion and adhesive tape.

Ninety-four punctures were made in seventy-four rabbits. The results are given in table 1 and summarized in table 2. In sixty-two cases the temperature did not rise above 41° C. This falls within the range of variability due to anaesthetics and operative injury, which I have shown to be between 36.9° to 40.9°C. and cannot be considered as hyperthermia brought about by injury to or stimulation of a specific "heat center." Since the variability range due to incidental factors is large and since occasional temperatures above 41.4°C. are met with in normal and anaesthetized rabbits, it seems reasonable to include only those registering 41.5°C. and above in the cases of hyperthermia. This would give seventy-four cases of normal temperature to twenty of hyperthermia in the above mentioned table.

The area and location of the brain lesion varied. This was determined by means of transverse sections of the brains hardened in formalin. In thirty-seven cases of normal temperature the caudate nucleus was distinctly injured. Figure 1 gives specimen sections through the line of puncture in two of these cases. The injury to the caudate nucleus cannot be questioned. Chart 1 gives curves of the temperature following these and other similar punctures. A comparison with the curves of normal and anaesthetized rabbits shows that these temperature variations could be accounted for by other means than injury to special "centers."

In five of the cases of normal temperature following puncture of the caudate nucleus the injury was limited to the caudate nucleus; in five it was extensive with infiltration involving large areas. In the remaining cases the lesion was well defined but included with the caudate nucleus other parts as the internal capsule, lenticular nucleus, optic thalamus or infundibulum.

In eighteen cases of normal temperature there was no evident injury to the caudate nucleus (fig. 2, chart 2). In fifteen cases of normal temperature injury to the caudate nucleus was questionable as the puncture penetrated the lateral ventricle and therefore merely touched the medial edge of the caudate nucleus. They are in the table in a separate column as uncertain (fig. 3, chart 3).

In only twenty cases of punctures was a distinct hyperthermia beyond the range of variability obtained. Seven of these showed no injury to the caudate nucleus. Figure 4 is a transverse section through the point of puncture of one showing a clear line between the hemi-

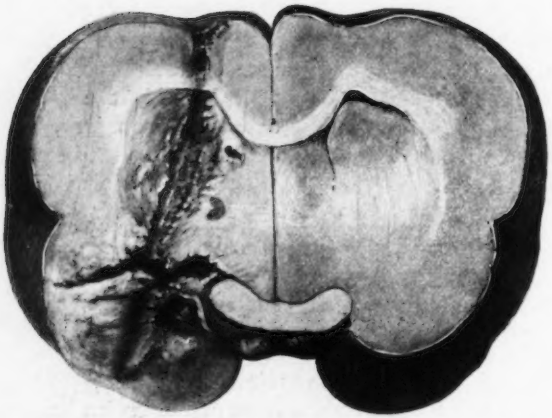

Fig. 1 a. Section of brain of rabbit 40 showing puncture through caudate nucleus; followed by normal temperature.

Fig. 1 b. Section of brain of rabbit 79; the puncture not wholly in one plane indicated by dotted line.

spheres with no injury to the caudate nucleus. Chart 4 gives the temperature curve of the same. Six cases had lesions (four extensive) which involved the caudate nucleus (fig. 5, chart 5). Six cases were doubtful.

Table 2 shows that 85 per cent of the punctures involving the caudate nucleus failed to produce a rise in temperature above 41.5°C. Only 15 per cent gave distinct hyperthermia.

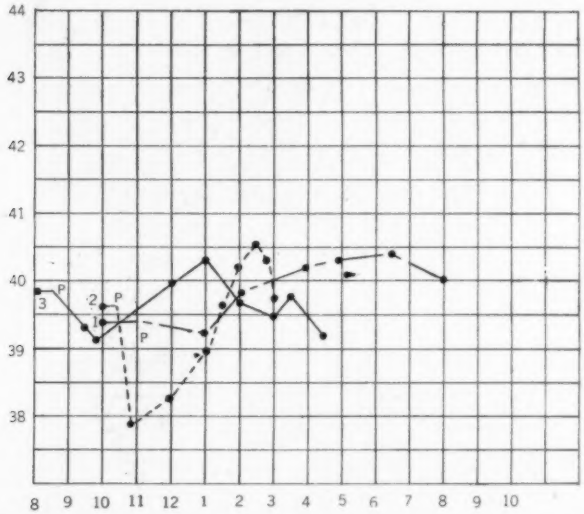

Chart 1. Specimen curves of normal temperature following puncture of caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 59; *2*, rabbit 79; *3*, rabbit 27, *4*, rabbit 40. *P*, Puncture.

In the above experiments all cases of hyperthermia were associated with excessive muscular movements often taking the form of clonic convulsions. Calorimetric measurements of the heat production were not taken but from the usual rise accompanying muscular exercise it seems reasonable to assume that the violent movements were sufficient to account for the hyperthermia. Exact data on this phase of the subject will be obtained in later investigations.

In another series of experiments punctures were made with a "heating and cooling cylinder" similar to the one used by Barbour (38) for the purpose of heating and cooling the caudate nucleus. The needle

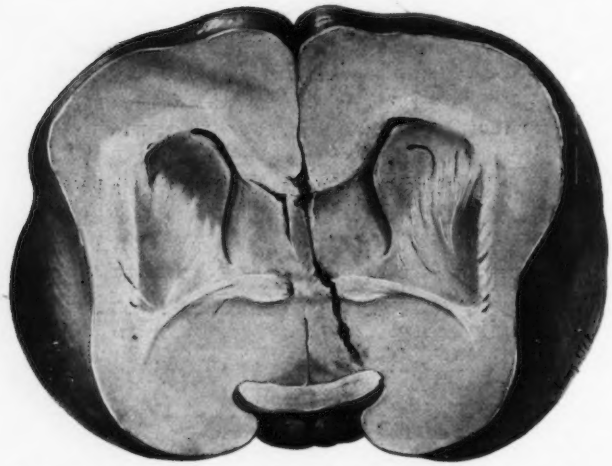

Fig. 2. Section of brain of rabbit 67; showing puncture line between the hemispheres with no injury to the caudate nucleus. A normal temperature followed.

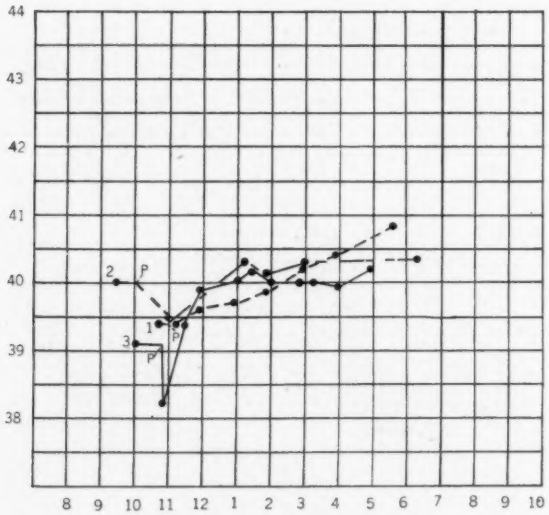

Chart 2. Specimen curves of normal temperature following punctures with no injury to the caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 67; *2*, rabbit 73; *3*, rabbit 17. *P*, Puncture.

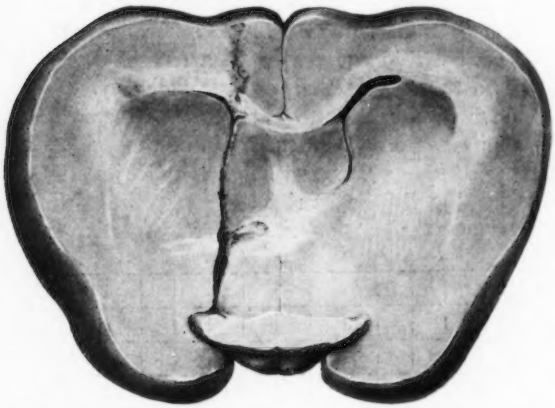

Fig. 3 a. Section of brain of rabbit 71 showing puncture through lateral ventricle with possible injury to caudate nucleus; followed by hyperthermia.

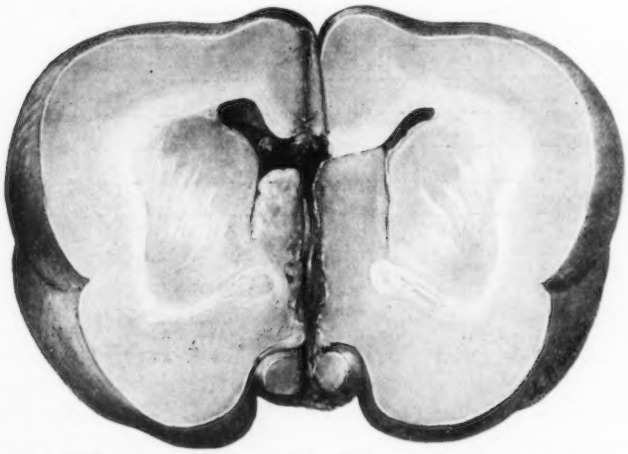

Fig. 3 b. Section of brain of rabbit 47 showing puncture through lateral ventricle; followed by normal temperature.

was 3 to 4 mm. in diameter instead of 1 mm. as in the first series; only five of the thirteen cases showed hyperthermia which was, in every case, preceded and accompanied by violent muscular movements and convulsions; six died within the course of a few hours. The cause of sudden death in these and other cases is being investigated further. While the above results seem to indicate that hyperthermia does in certain cases follow puncture of the brain of the rabbit, it cannot be said that the rise in temperature depends on injury to the caudate nucleus nor can the rise be ascribed to any other definite "center" since there is no apparent correlation between the location of the

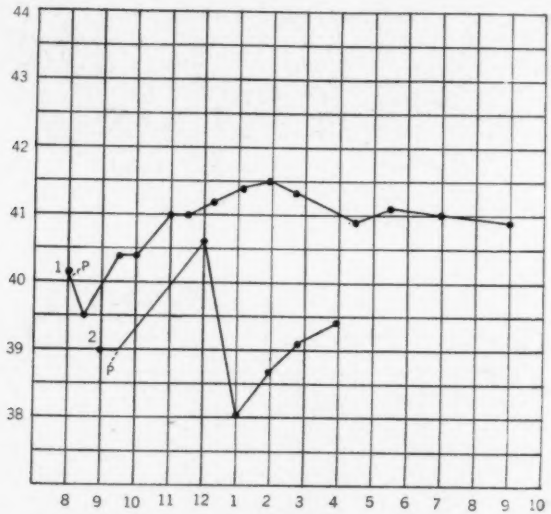

Chart 3. Specimen curves of temperature variations following punctures in the lateral ventricle. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 71; *2*, rabbit 47. *P*, Puncture.

lesion and the occurrence of hyperthermia. It can be concluded, in fact, that injury to the caudate nucleus or other alleged "heat centers" and "puncture fever" bear no close relation to each other.

B. Application of pyretics and antipyretics. The caudate nucleus was heated and cooled according to the method of Barbour (38) and of Hashimoto (21) by means of a metal cylinder through which hot or cold water could be passed at will. Heating caused a fall in temperature of 0.2° to 1.4°C. Cooling a rise of 0.5° to 1.6°C. Table 3 gives the

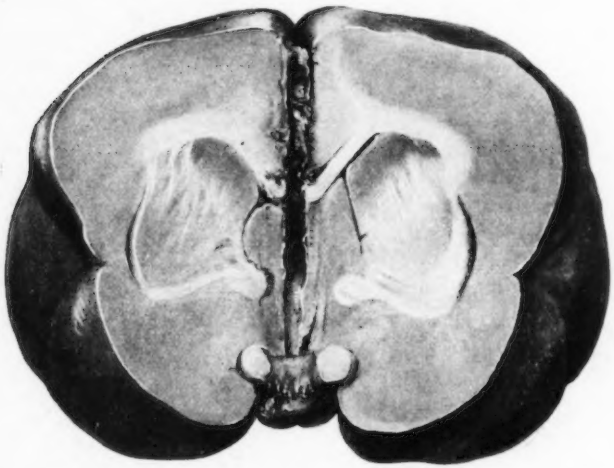

Fig. 4. Section of the brain of rabbit 22 showing puncture line between the hemispheres with no injury to the caudate nucleus. Hyperthermia followed the puncture.

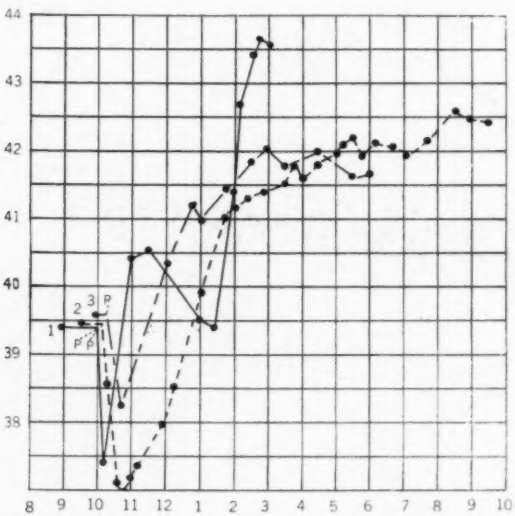

Chart 4. Specimen curves of hyperthermia following punctures with no injury to the caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 22; *2*, rabbit 10; *3*, rabbit 29. *P*, Puncture.

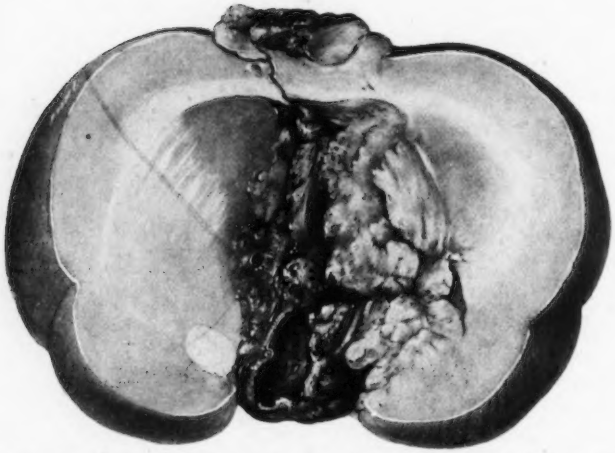

Fig. 5 a. Section of brain of rabbit 61 showing extensive lesion involving caudate nucleus; followed by hyperthermia.

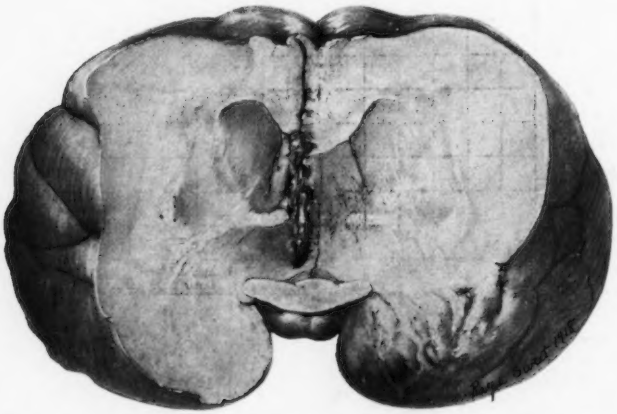

Fig. 5 b. Section of brain of rabbit 51 showing slight injury to caudate nucleus; followed by hyperthermia.

results. They accord with those of Barbour (38) and of Hashimotor (21) but might conceivably be due to an indirect or direct effect on the vasomotor centers in the medulla. The fact that heating and cooling the medulla (experiments to be described later) gave similar results tends to show that this may be the case.

An attempt was made to apply drugs to the caudate nucleus, as Barbour and Wing (18) had done, by injecting into the puncture hole. Barbour states that there was often an overflow of ventricular fluid and drug. The same difficulty was met in my experiments. In every

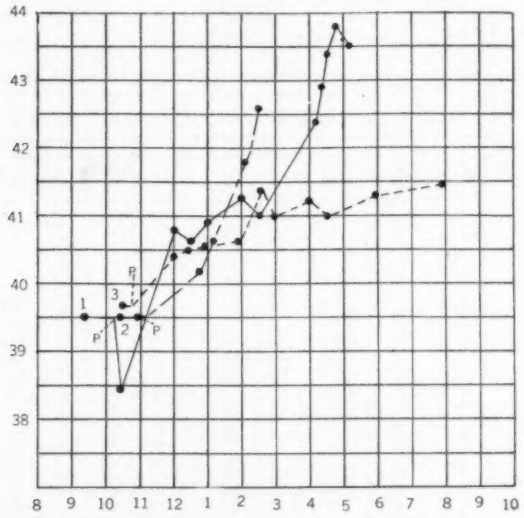

Chart 5. Specimen curves of hyperthermia following puncture of the caudate nucleus. Ordinates, degrees Centigrade; abscissae, time in hours. *1*, Rabbit 20; *2*, rabbit 61; *3*, rabbit 51. *P*, Puncture.

case the pressure of the fluid was sufficient to render it very doubtful whether any of the drug reached the brain tissue, especially the caudate nucleus. A more exact method of applying drugs to parts of the brain below the cortex should be devised.

C. Removal. With aseptic precautions a large area of the brain was exposed by removing the skull with a trephine and bone forceps. Bleeding was stopped by pressure with sterile cotton. The cortex was lifted off with a large curette, thus leaving the caudate nuclei clearly exposed to view. They were then carefully removed with a

TABLE 1

Effect of puncture on the temperature of the rabbit

NUMBER OF RABBIT	DATE OF PUNCTURE	LESION	TEMPERATURE			REMARKS
			Before puncture	Maximum	Time of maximum	
	1916				*hours*	
1	November 13	No autopsy	39.4	38.4	7	
2	November 17	Lateral ventricle, *caudate nucleus doubtful*	39.3	39.2	5	
3	November 21	Lateral ventricle, optic thalamus, infundibulum, *caudate nucleus doubtful*	38.5	39.5	8	
3	November 25	Same as on 21st	39.4	40.1	7	
3	November 27	Left lenticular nucleus	39.4	40.2	6	
4	November 30	Lateral ventricle, optic thalamus, infundibulum	39.8	40.1	4	
4	December 3	Lateral ventricle, optic thalamus, infundibulum	39.9	40.7	3	
7	December 1	Lateral ventricle, *caudate nucleus*	40.1	39.9	3	
7	December 3	Lateral ventricle, *caudate nucleus*	39.1	40.45	5	
8	November 17	Lateral ventricle, *caudate nucleus* doubtful	39.5	40.6	2	
8	November 20	Lateral ventricle, *caudate nucleus* doubtful	39.6	40.1	$2\frac{1}{2}$	
8	November 21	Lateral ventricle, *caudate nucleus* doubtful	39.6	40.0	1	
8	November 24	Lateral ventricle, *caudate nucleus* doubtful	39.1	39.5	3	
10	December 19	Between hemispheres, optic chiasma	39.4	*42.2*	7	
14	December 30	*Caudate nucleus;* lenticular nucleus	39.1	41.1	6	
14	December 31	*Caudate nucleus;* lenticular nucleus	39.1	40.9	6	
15	December 30	Lateral ventricle, infundibulum	39.2	*41.5*	$4\frac{1}{2}$	
16	December 22	Lateral ventricle, optic thalamus, extensive infiltration, caudate nucleus doubtful	38.8	40.6	$6\frac{1}{2}$	

16	December 29	Same as on 22d	38.7	39.5	1½	
16	December 30	Same as on 22d	39.2	*41.5*	1½	
16	December 31 *1917*	Same as on 22d	40.7	39.7	1	
17	January 25	Between hemispheres, infundibulum	39.1	40.2	3	
17	January 26	Between hemispheres, infundibulum	39.3	40.3	5	Died on day of operation
18	January 25	No autopsy	39.4	40.1	10	
18	January 26	No autopsy	39.3	*43.0*	5	Convulsions
18	January 27	No autopsy	39.5	39.2	6	Died on day of puncture
19	January 25	Lateral ventricle, optic thalamus, infundibulum, caudate nucleus doubtful	39.5	41.4	7	
19	January 26	Same as on 25th	40.3	41.0	7	
19	January 27	Same as on 25th	39.5	40.4	3	
20	January 31	Extensive infiltration involving caudate nucleus, optic thalamus, infundibulum	39.5	43.8	7	Moved about continually and rapidly. Tremors. Died on day of puncture
21	January 30	Same as no. 20	39.6	41.3	8	
22	January 29	Between hemispheres, infundibulum, optic chiasma	39.4	*43.65*	5	Constant movement and tremors. Died on day of puncture
23	February 3	Lateral ventricle, infundibulum, optic thalamus	39.5	39.5	5	
23	February 5	Lateral ventricle, infundibulum, optic thalamus	39.6	41.1	9	
23	February 6	Lateral ventricle, infundibulum, optic thalamus	40.1	37.1	3	Died on day of puncture
25	February 7	Extensive optic thalamus, infundibulum, *caudate nucleus*	39.7	39.3	1½	Died on day of puncture
26	February 7	Lateral ventricle, optic thalamus, *caudate nucleus*	39.7	41.5	6	Died on day of puncture

TABLE 1—*Continued*

NUMBER OF RABBIT	DATE OF PUNCTURE	LESION	TEMPERATURE			REMARKS
			Before puncture	Maximum	Time of maximum	
	1917				*hours*	
27	February 8	Lateral ventricle, infundibulum, optic thalamus, *caudate nucleus*	39.6	40.5	4	Died on day of puncture
28	February 10	Lateral ventricle, infundibulum, optic thalamus, *caudate nucleus doubtful*	39.3	41.0	4	Moved continually, died on day of puncture
29	February 13	Septum pellucidum, infundibulum	39.5	*42.0*	5	
29	February 15	Left side, through cortex only	37.6	37.2	5	Convulsions. Died on day of puncture
33	February 19	Lateral ventricle, whole *corpus striatum*	39.5	41.4	6	Convulsions. Died on day of puncture
35	February 20	Lateral ventricle whole *corpus striatum*	39.8	41.3	7	
36	February 21	Lateral ventricle, *caudate nucleus* infundibulum	39.6	41.4	7	
37	February 22	Lateral ventricle, *caudate nucleus*, lenticular nucleus	39.2	40.5	8	
38	February 28	Between hemispheres, optic chiasma	39.6	41.7	10	Continual movement. Died on day of puncture
39	February 28	Extensive, caudate nucleus, optic thalamus, infundibulum	36.6	41.0	10	
39	March 1	Same as on February 28	41.0	39.7	8	Died on day of puncture
40	March 1	Lateral ventricle, caudate and lenticular nucleus	39.8	40.3	10	Died on day of puncture
41	March 1	Lateral ventricle, caudate nucleus, infundibulum optic thalamus	39.4	40.3	11	

41	March 2	Lateral ventricle, caudate nucleus, infundibulum optic thalamus	40.3	41.3	3	
42	March 5	Lateral ventricle, infundibulum, optic thalamus	38.9	40.4	7	Convulsions; died on day of puncture
43	March 5	Lateral ventricle caudate nucleus, optic chiasma	39.6	40.5	5	
43	March 6	Lateral ventricle, caudate nucleus, infundibulum	39.7	*43.2*	8	Frantic movements. Died on day of puncture
44	March 6	Lateral ventricle, *caudate nucleus doubtful*	39.2	38.8	4	Died in four hours
45	March 7	Lateral ventricle, *caudate nucleus,* infundibulum, optic thalamus, extensive	39.8	*41.8*	4	
46	March 10	Lateral ventricle, *caudate nucleus,* infundibulum, optic thalamus, extensive	38.5	*42.0*	3½	Frantic movements and convulsions. Died on day of puncture
47	March 12	Lateral ventricle, *caudate nucleus, doubtful,* infundibulum, optic thalamus, extensive	39.0	39.4	6	
48	April 30	Lateral ventricle, *caudate nucleus, doubtful,* infundibulum	39.8	*42.6*	10	Frantic movements, convulsions; died on day of puncture
49	May 2	Lateral ventricle, *caudate nucleus*	39.0			Died in two hours
50	May 3	Lateral ventricle, *caudate nucleus,* infundibulum, optic thalamus	39.6	39.8	6	
51	May 4	Lateral ventricle, *caudate nucleus,* slight	39.6	*41.5*	9	
52	May 5	Lateral ventricle, *caudate nucleus,*	39.2	40.8	1	
53	May 6	Lateral ventricle, *caudate nucleus,* infundibulum, optic thalamus	39.1	40.3	5	
53b	May 6	Lateral ventricle, *caudate nucleus*	39.2	40.1	4	Convulsions. Died on day of puncture
54	April 30	Lateral ventricle, caudate nucleus, optic thalamus	38.8	39.5	7	

TABLE 1—*Continued*

NUMBER OF RABBIT	DATE OF PUNCTURE	LESION	TEMPERATURE			REMARKS
			Before puncture	Maximum	Time of maximum	
	1916				*hours*	
55	April 30	Lateral ventricle	39.9	41.2	3	Sick
56	May 1	Lateral ventricle, infundibulum, optic thalamus	40.0	*42.2*	2	Constant rapid movement
57	May 1	Lateral ventricle, caudate nucleus, optic thalamus, infundibulum	40.1	41.2	5	Died on day of puncture
58	May 2	Between hemispheres	39.3	40.5	4	
59	May 3	*Caudate nucleus*, internal capsule, optic thalamus	39.5	40.6	3	
60	May 6	*Caudate nucleus* slightly, lateral ventricle	38.8	40.8	6	
61	May 6	*Caudate nucleus* extensive, infundibulum	39.5	42.55	4	Continual movement, convulsions; died on day of puncture
62	May 7	*Caudate nucleus doubtful*, corpus callosum	39.5	*41.5*	4	
63	May 7	*Caudate nucleus*, lenticular nucleus	39.3	40.0	7	
64	May 8	Between hemispheres, infundibulum	39.3	41.1	5	
65	May 8	To corpus callosum	39.5	41.0	7	
66	May 8	Extensive, caudate nucleus, optic thalamus, infundibulum	39.6	40.9	3	Died on day of puncture
67	May 8	Between hemispheres to optic chiasma	39.5	40.3	4	
68	June 19	*Caudate nucleus* doubtful, infundibulum	40.0	*42.3*	6	Very restless
68	June 20	*Caudate nucleus* doubtful, infundibulum		*42.0*	2	
69	June 21	*Caudate nucleus*, lateral ventricle, infundibulum, optic thalamus	39.65	38.8	3	

70	June 22	*Caudate nucleus*, lateral ventricle, infundibulum, optic thalamus	39.4	40.2	5	Slight convulsions. Died on day of puncture
71	July 3	*Caudate nucleus* doubtful, lateral ventricle, optic chiasma	40.0	41.5	5	
72	July 5	Lateral ventricle, optic thalamus	39.0	40.5	7	
73	July 12	Optic thalamus, fornix, lenticular nucleus	40.1	40.8	6	Died on day of puncture
74	July 14	Optic thalamus, fornix, infundibulum	39.5	*42.0*	6	
75	July 15	Optic thalamus, fornix, lenticular nucleus	40.0	40.6	6	
76	July 20	Caudate nucleus, lenticular nucleus, internal capsule	39.5	40.8	7	
77	July 25	Extensive, caudate and lenticular nuclei, optic thalamus, infundibulum	30.2			Died in convulsions in 1½ hours
78	July 26	Lateral ventricle, caudate nucleus slightly, optic thalamus, infundibulum	39.3	40.4	3	Died on day of puncture
80	August 2	No autopsy		40.3	5	
81	August 5	Between hemispheres		41.4	5	
82	August 6	Between hemispheres		39.0	5	

TABLE 2

Summary of effect of puncture on the temperature of the rabbit

LESION	NUMBER OF PUNCTURES			
	Hyperthermia		NORMAL TEMPERATURE	
	Above 41.5°C.	Above 41.°C.	Below 41.5°C.	Below 41.°C.
Involving caudate nucleus	*6*	13	*37*	30
Not involving caudate nucleus	*7*	11	*18*	14
Possible injury to caudate nucleus	*6*	7	*15*	14
No autopsy	1	1	4	4

TABLE 3

The effect of heating and cooling the caudate nucleus

NUMBER OF RABBIT	DATE	TEMPERATURE			DURATION OF PASSAGE OF WATER
		Before passage of water	After passage of water, 45-50°C.	Amount of change °C.	
	1917				*hours*
100	November 14	40.5	39.4	−1.1	1½
105	November 21	39.9	38.5	−1.4	1
120	December 10	41.2	39.4	−1.8	2½
	1918				
123	January 30	41.1	40.5	−0.6	1
	January 31	40.5	40.9	+0.4	½
126	February 2	38.7	38.5	−0.2	1
	February 3	38.5	38.7	+0.2	½
			Water 15 −20°C.		
	1917				
105	December 21	40.5	39.9	−0.5	1
	1918				
123	January 30	41.0	41.5	+0.5	½
	January 31	40.9	41.5	+0.6	1
126	February 2	38.9	40.5	+1.6	2

small curette. The skin on the head was then replaced and fastened together and the wound bandaged with cotton and flexible collodion and adhesive tape. The rabbits survived the operation several days. Some were killed on the third day.

Eighteen operations were performed and in every case a normal temperature was maintained. Careful autopsies by means of transverse sections of the brains hardened in formalin were made. In seven brains no trace of the caudate nuclei remained, in ten a portion 1 mm.

or less in diameter of the posterior tip was intact but could have had no connection with any other portion of the brain nor with the cord.

Similar experiments were made on pigeons. Since the corpus striatum makes up the major part of the forebrain, both cerebral hemispheres were removed. The same results were obtained as for rabbits, that is, a normal temperature was maintained subsequent to the operation in every case. Physiological behavior as well as autopsy findings indicated that the cerebral hemispheres had been completely removed.

These results indicate that a normal body temperature in rabbits and pigeons can be maintained without the aid of the caudate nucleus of the corpus striatum.

SUMMARY

1. Seventy-eight per cent of all the punctures failed to produce an abnormally high temperature. Of the 22 per cent of cases in which hyperthermia was obtained, only one-third showed injury to the caudate nucleus. Approximately one-half of the punctures were distinctly through the caudate nucleus; 85 per cent of these, however, were not followed by hyperthermia.

2. Heating the caudate nucleus caused a slight fall in temperature, cooling a slight rise. In this respect the results agree with those of Barbour.

3. After removal of the caudate nucleus in rabbits and the cerebral hemispheres in pigeons, a normal body temperature was maintained.

CONCLUSIONS

The corpora striata are not essential for the maintenance of a constant body temperature since their puncture in rabbits or their removal in rabbits and pigeons does not alter the normal temperature.

The existence of special "heat centers" in the brain is therefore not confirmed.

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THE NURSING MOTHER AS A FACTOR OF SAFETY IN THE NUTRITION OF THE YOUNG[1]

E. V. McCOLLUM AND N. SIMMONDS

From the Biochemical Laboratory of the School of Hygiene and Public Health of the Johns Hopkins University, Baltimore

Received for publication April 18, 1918

THE ESSENTIAL CONSTITUENTS OF AN ADEQUATE DIET

Since the solution of the main problems involved in the successful feeding of simplified diets which consisted of purified substances, progress in the advancement of our knowledge of nutrition has been rapid. It has become evident that among the thousand or more chemical substances which occur in animal and plant tissues it is essential that the diet of the mammal shall contain only the following:

Sixteen or seventeen amino-acids which result from the digestion of the complete proteins;[2] the carbohydrate glucose or one of its polysaccharides (starch, dextrine, etc., or other sugar which in the body is convertible into glucose); probably nine inorganic elements in the form of suitable compounds (Ca, Mg, Na, K, Cl, P, I, Fe, S), and two as yet chemically unidentified dietary essentials, fat-soluble A[3] and water-soluble B. Such a mixture may be capable of supporting normal nutrition throughout the life of an animal beyond the weaning period.

[1] A few of the experiments described in this paper were carried out by the authors at the Wisconsin Experiment Station.

[2] So far as has been definitely established all the amino-acids which the "complete" proteins yield on hydrolysis must with the exception of glycocoll be supplied in the food mixture. The suggestive experiments of Hopkins (1) seem to indicate that several of the aliphatic amino-acids are dispensable from the diet, but the feeding periods were, in our opinion, too brief to make the results conclusive.

[3] Although it has not been found possible to successfully nourish animals on diets free from lipoids, there is much evidence that not only fats and lecithins, but the other complex lipoids as well can be synthetically produced by the animal tissues (2). It seems probable that if it were possible to supply fat-soluble A without at the same time adding fats, young animals could be satisfactorily nourished on lipoid-free diets.

The terms fat-soluble A and water-soluble B were introduced by McCollum and Kennedy to designate two chemical substances, or so far as could be determined at that time, possibly groups of substances, both of which are indispensable in the diet (3). Subsequent developments have made it clear that at least in the case of the water-soluble B but one substance indispensable for the maintenance of physiological well being is involved (4). In the case of the dietary factor fat-soluble A, it is possible that more than a single indispensable substance is present in the simplest food preparations which furnish it but there is not the slightest evidence that there is more than one. Fat-soluble A is the dietary factor of unknown chemical nature, the absence of which from the diet leads to the development of a peculiar condition of the eyes. The eyelids and tissues surrounding the eyes swell so that the eyes cannot be opened or are opened with difficulty. There is inflammation of the cornea, and if the missing dietary essential is not promptly supplied, permanent blindness ensues. This disease is a type of xerophthalmia (5). Butter fat, milk and the fats of egg yolk are the best sources of the substance, fat-soluble A, but it is also present to the extent of about three times the food requirements of the growing animal in the leaves of such plants as spinach, celery (tops), alfalfa and other leaves which are not fleshy and which dry easily when separated from the plant. This is shown by the fact that 30 per cent of one of these leaves in the diet supplies enough of this substance to maintain an animal in a normal condition, provided the rest of the diet is properly constituted (6). Fleshy leaves such as the cabbage, which are in some degree modified as storage organs, contain proportionately less of the substance, fat-soluble A. Seeds and seed products contain in general much less of the substance than do the leaves. This difference in the content of different foods in the dietary essential, fat-soluble A, depends upon the extent to which the foodstuffs consist of cellular elements as contrasted with reserve food materials (protein, starch, sugars, fats and inorganic salts). The substance is associated with the germ and with the limited areas of the seed which consist of cellular elements, rather than with the endosperm (7).

The second dietary essential of unknown chemical nature, water-soluble B, is much more abundant in nature than is fat-soluble A. It too is associated more abundantly with the cells of the animal and plant tissues rather than with the reserve food supply of the seed. A lack of this substance in the diet leads to the development of a condition of polyneuritis which in man is known as beriberi. There is

almost conclusive evidence that the preparations which contain this substance in concentrated form contain but a single indispensable chemical substance of unknown nature. This view is borne out by the fact that beriberi and the type of xerophthalmia of dietary origin are the only diseases referable to faulty diet, for which "curative" substances exist. Other diseases, especially scurvy and pellagra, have been referred by Funk (8) and others to the group of so-called deficiency diseases, in the same sense as the syndromes beriberi and xerophthalmia, i.e., the assumption is made that for each of them there exists a "curative" substance.

Our knowledge concerning the degree to which the diet of the higher animals can be simplified without interfering with their normal development, is the result of a long series of feeding experiments with purified foodstuffs carried out by McCollum and Davis (9). It has a scientific interest in that it makes clear the great extent of the synthetic power of the animal body, but a very much greater value in that it afforded the key to the solution of the greatest problem of the human race, viz., the problem of approximating the optimum in the character of the food which we consume. For half a century the energy and protein content have been the criteria by which dietitians have attempted to judge the quality of food mixtures. In a land of plenty, where a wide variety of foodstuffs is available, dairy products, meats and the produce of the vegetable garden, as well as the staple cereals and potatoes are within the reach of even the poorest people, it is not strange that the inadequacy of such a basis for the estimation of the quality of rations should long escape criticism. Only in the field of animal production, where rations monotonous in character and restricted as to source to one, two or three foodstuffs, were fed week after week, did it become apparent that the data afforded by chemical analysis failed to disclose the quality of a food mixture as made manifest by its power to induce rapid growth in the young, vigor and high fertility in the adult, and the capacity to produce strong offspring and an abundance of milk for their nutrition. That this fact was recognized by shrewd animal husbandrymen is made evident by the fact that although standard works on the feeding of farm animals continue to the present day to discuss food values on the basis of crude protein content, energy value and digestibility, agricultural experiment stations have for years been testing by feeding experiments the value of this versus that protein concentrate as a supplement for each of the more important feeds grown on the farm. If the proteins from one source were as good as

those from another, and the digestibility were equally high, such comparative feeding trials should be unnecessary and the feeding value of several mixtures which would give similar results when subjected to chemical analysis should be equal. This, however, is not the case. Feeders have long recognized the superiority of milk and buttermilk over any of the by-products of the milling industry, as supplements to rations derived from seeds and seed products.

The nature of the dietary deficiencies of the seeds of plants. Once in possession of the knowledge concerning just what factors operate to make an adequate diet, McCollum and Davis, McCollum, Simmonds and Pitz, and McCollum and Simmonds were able to show by the systematic feeding of a single natural foodstuff, as wheat (10), maize (11), rolled oats (12), rice (13), wheat germ (14), pea (6), navy bean (15), kaffir corn (16), (17), rye and barley (17), with single and multiple purified food additions, just what kind of deficiencies are responsible for the failures which had long been known to result from the feeding of certain diets which were greatly restricted as to source. The soy bean has been studied by Daniels and Nichols (18) and also by Osborne and Mendel (19), and the cottonseed by Richardson and Green (20).

It was a great surprise to find that such a mixture of seeds as whole wheat, rolled oats, whole corn, unpolished rice, etc., when fed as the sole source of nutriment fail utterly to induce any growth in young animals or to long maintain life. The reason for this we have shown in the papers cited.

These investigations made it clear that the seeds as a class are deficient in the same respect, viz., they are all too poor in three inorganic elements, calcium, sodium and chlorine, to permit an animal to grow. It was shown that each of these seeds is not enhanced by the addition of any other inorganic elements than those mentioned (21). Each of these seeds, with the exception of millet seed (22), is too low in its content of the fat-soluble A to maintain an animal in a good state of health over a long period, and the quality of the protein in each is of relatively low biological value and must be supplemented by the addition of protein before growth at the maximum rate can be secured. The seeds are therefore to be classed together as a distinct group of natural foods having the same limitations from the dietary standpoint. For an appreciation of the problems relating to the diet during lactation which we present in this paper, it is essential that the following facts be fully appreciated:

1. Young animals cannot grow when limited to a single seed or mixture of seeds as their sole source of nutriment, with no accidental supply of mineral salts in the drinking water.

2. Young animals cannot grow when fed a single seed or mixture of seeds, even though the latter is supplemented with purified protein and a fat containing fat-soluble A. The inorganic content is the first limiting factor and sodium, chlorine and calcium must be added before growth becomes possible.

3. The proteins of the seeds, and their content of fat-soluble A, as well as all other dietary factors, are of such a value as will permit young animals to grow for a considerable time, and remain in apparent good health when the diet consists of one or more seeds, supplemented with the necessary inorganic salts. On such a diet faulty nutrition is first observed only after the lapse of a considerable time.

DIFFERENCES IN THE COMPOSITION OF THE MILK AS THE RESULT OF THE QUALITY OF THE DIET OF THE MOTHER

The extent to which the maternal organism through the secretion of the mammary gland can serve as a factor of safety for the suckling, is still very little understood. It is well known that the proteins of milk are of distinctly higher quality for the promotion of growth than are those of the vegetable foods generally. This is shown by the fact that young animals grow better on 6 or 7 per cent of milk protein (23) than on higher intakes of plant proteins (24). In respect to the protein factor, therefore, the maternal organism selects certain of the amino-acids from among the digestion products of the food and presents them in the milk for the nutrition of the young in such proportions as make possible a very efficient transformation into the body proteins of the young. She makes from her large intake of protein of rather poor quality a smaller output of protein of exceptional biological value in the milk

The importance of this service of the mother to the young in supplying it a protein mixture suitable for efficient utilization for growth, will doubtless depend upon the character of the mother's food. It is well known that certain amino-acids greatly stimulate metabolism because of their "specific dynamic action" (25), and lead to increased heat production and increased carbon dioxide output. Beyond a certain limit, not well defined, this would be of course a useless waste of energy and would tend to interfere with the formation and with the

storage of new tissue. It is like keeping the furnace going in warm weather. In former papers we attempted to throw light on the question as to whether the young animal is better off with the minimum supply of protein of high biological value, necessary for normal growth, than it is with food proteins of low value for growth when the latter are fed at such high planes of intake that the same biological value for growth would be reached or surpassed. Wheat proteins (24) were employed in the one series of experiments and milk proteins in the other (23). The animals were able to grow better on 6 to 8 per cent of milk proteins than on any plane of wheat proteins up to over 40 per cent of the food mixture. The results were not considered conclusive, however, in giving an answer to the question in point because it appears that there is something detrimental about the wheat products which may have depressed the vitality of the animals. Final deductions cannot be drawn until these experiments are repeated using proteins of low value but without detrimental qualities other than such as may come from the disposal by the organism of the excess of useless amino-acids which cannot, because of certain essential ones being nearly absent, be built up into tissue proteins.

The lactating mother certainly concentrates in the milk she produces much, if not all, of the dietary essential fat-soluble A which she ingests in her large intake of leaf and seed products, and thus enable the young to obtain a much larger amount of this substance than it could possibly obtain by eating the food taken by the mother, because of the limited capacity of its digestive apparatus. The same is doubtless true of the second dietary essential of unknown chemical nature, water-soluble B, but this would seem not to be a matter of great significance since this substance is so abundant in most of the natural foods that the young could easily secure enough for its needs in the limited amounts of food which it could ingest.

Milk represents a very concentrated food from the standpoint of energy and protein values (although associated with much water), is easy of digestibility and is, when drawn by sucking, nearly free from bacteria. Milk is therefore a food, the elaboration of which involves the functioning of the mother as a protective agent in her relation to her offspring. There are further to be considered certain possible synthetic powers which the mammary tissue may possess, in which it may surpass the powers of the tissues of the growing young.

Milk sugar, the only carbohydrate of milk, is found nowhere except in the secretion of the mammary gland. It affords an example, there-

fore, of a special synthesis by the maternal organism for the nutrition of the young. Like examples of the exercise of a synthetic function by the mammary tissue in milk production are seen in the peculiar nature of the fatty acids of low molecular weight found in milk fats. These acids do not need to occur in the food of the lactating animal.

The fact that the mammary gland is able to synthesize the sugar lactose and certain of the fatty acids which are peculiar to the milk fats is suggestive of the possibility that these tissues may be able to effect the transformation of certain amino-acids into others in a manner not possible to the non-lactating animal or to the growing young. There is recorded in the literature an experiment by Osborne and Mendel (26) which was interpreted at the time it was carried out as constituting a demonstration of the ability of the mammary gland to synthesize the diamino-acid lysine, and as supporting the idea that certain transformations of amino-acids not possible to the immature tissues of the young may be effected by certain tissues under special conditions, such as for example in milk formation.

Owing to the fact that certain unwarranted assumptions were made at the time their experimental work was reported, there is much misconception as to the special rôle of the amino-acid lysine in nutrition. They employed diets which contained 28 per cent of "protein-free milk" and added gliadin as the sole purified protein. Osborne and Mendel (27) assumed that the gliadin employed "does not yield more than insignificant amounts of lysine" (p. 342), and interpreted their data as showing that "certain proteins, notably the gliadin of wheat, may supply the nitrogenous needs of an animal in maintenance, yet be entirely inadequate for the purposes of growth" (p. 328). They further state (p. 332), "we have succeeded in promoting growth at a normal rate when a maintenance ration containing gliadin as the sole protein was supplemented with lysine," and (p. 333) "the demonstration that the addition of lysine to the gliadin food serves to render this protein of wheat entirely adequate for the nitrogenous needs of growth is shown in chart 1, rat 1113, in the appendix, in which the surprising effect of this amino-acid addition is in strong contrast with the mere maintenance effect of the diet without the lysine," and further, "we believe that these feeding trials, in conjunction with our demonstration of the almost complete cessation of growth on diets containing only lysine-free proteins, furnish the first and only conclusive demonstration that lysine is indispensable for the functions of growth." On page 334, they further state "the animal organism apparently

cannot synthesize lysine, which is evidently not essential for maintenance in the sense of preservation of body weight, though it is, of course, impossible to say that when this amino-acid is missing, all functions are normally carried out."

These conclusions, we feel confident, are based upon unwarranted assumptions concerning the character of the food mixtures which these authors employed in their experimental work. It is desirable that so important a deduction as the differentiation between the requirements of the animal for maintenance as contrasted with growth, if unwarranted, should be clearly shown to be fallacious. We take this occasion, therefore, to offer a critique of certain of the conclusions of Osborne and Mendel, which are based upon experiments of such a character as to appear open to criticism only to those who, like ourselves, have studied closely the problems relating to the behavior of animals fed upon diets consisting of isolated and carefully purified foodstuffs, and simplified as far as is possible, consistent with the normal nutrition of an animal.

The quotations above refer to the relation of the amino-acid lysine to the nutrition of the growing young animal. Osborne and Mendel employed a diet of similar composition to that discussed above in its relation to maintenance as contrasted with growth, and describe what they interpreted to be a pregnancy and successful lactation period in a rat (26). Four young were brought to the age of twenty-three days during which they grew at approximately the normal rate, while the mother was restricted to the "gliadin food," which was assumed to contain but an insignificant amount of lysine but to be otherwise complete as a source of amino-acids. They believed that lysine was not necessary for the long continued maintenance of an animal (27), but indispensable for growth, and the conclusion seemed warranted that the mother, taking supposedly lysine-free food and producing during gestation four young, and during lactation milk which was capable of inducing nearly normal growth in the young, was able through the special powers possessed by the mammary tissues to synthesize the amino-acid lysine for the formation of normal milk proteins. Casein and lactalbumen both contain 7 to 8 per cent of lysine.

The "gliadin food" in the lactation experiments consisted of carefully purified gliadin, 18 per cent; "protein-free milk," 28 per cent; starch, lard and agar-agar (26). Gliadin has been shown in a reinvestigation by Osborne to have been erroneously assumed to be lysine-free. Furthermore, for reasons explained later, we are convinced that

the dietary properties of "protein-free milk" were not fully understood by these authors. They believed at that time that the gliadin was practically free from the amino-acid lysine and they minimized the possible importance of the nitrogenous components of the "protein-free milk" as a source of amino-acids and drew the conclusion that the mother was effecting a synthesis of this particular protein cleavage product (lysine), since the young were unable to grow on the diet of the mother after the period when they may safely be weaned. Owing to the important deductions drawn from these experiments, certain erroneous assumptions made regarding the quality of the diet used in the experimental work referred to, should be pointed out. Other experiments were reported in the same paper in which steady loss of weight followed when the diet consisted of starch, sucrose, lard, gliadin, salts and agar-agar, and such losses were regained when "protein-free milk" or feces were supplied. This result may have been due to the addition of both the dietary essentials, fat-soluble A and water-soluble B, which were lacking in the purified diet and whose significance was not at that time appreciated, as well as possibly to the supplementary value of the nitrogenous compounds, e.g., lysine, of the "protein-free milk" or feces.

The later discovery by Osborne, Van Slyke, Leavenworth and Vinograd (28) that there is about 1.34 per cent of lysine in the most carefully purified gliadin, and the lack of evidence that "protein-free milk" does not supply lysine to some extent, renders very problematical the correctness of the conclusions concerning the ability of the maternal organism to synthesize lysine through the medium of the mammary gland for the maintenance of the species, as contrasted with the inability of the young animal to effect the same synthesis for its own preservation during growth. The food of the mother contained lysine and the amount of this amino-acid available for the synthesis of milk proteins depended upon the capacity of the mother to consume and digest food protein, poor in this complex, above her own body needs.

"Protein-free milk" contains about 0.76 per cent of nitrogen and a diet containing 28 per cent of this substance derives 0.2128 gram of nitrogen per 100 grams of ration from this source. When a food mixture is prepared, as were many of those employed by Osborne and Mendel, by the combination of 18 per cent of purified protein with 28 per cent of "protein-free milk," and the remainder of the food mixture was composed of nitrogen-free substances, the resulting food mixture

derives 93 per cent of its total nitrogen from purified protein and 7 per cent from the "protein-free milk." The proportion of the total nitrogen of the diet which comes from the uncharacterized forms in "protein-free milk" rises, of course, when the amount of purified protein is decreased. In some of their experiments in which the purified proteins were fed as low as 2 per cent of the food mixture, the assumption was made that this purified protein furnished the sole significant nitrogen from the standpoint of nutrition, but in reality 63 per cent of the total nitrogen of the diet was derived from the "protein-free milk" (29). McCollum and Davis have presented evidence which indicates that this is a source of amino-acids (30). This so-called "non-protein" nitrogen has been consistently ignored as being of no biological value, but that Osborne and Mendel now appreciate its significance is shown by a recent publication (31).

Munk (32) stated that about $\frac{1}{16}$ of the total nitrogen of milk is in the form of "non-protein" nitrogen. By this he meant that it could not be precipitated by such reagents as alcohol, tannin, copper hydroxide, etc. Osborne and Mendel state, "since our protein-free milk powder was equal to 50 per cent of the total solids of the milk, it should, *if Munk's statements are correct*,[4] contain 0.48 per cent of non-protein nitrogen, thus leaving at the most only 0.28 per cent of protein nitrogen equal to 1.69 per cent of protein. Since 100 grams of the food mixture employed in our experiments contained 28.2 grams of protein-free milk powder, *we can assume*[4] that at most the food pastes thus made contained only 0.48 per cent of milk protein" (33). They further stated in commenting upon the composition of their diet of gliadin, "protein-free milk," starch, lard and agar-agar: "such analyses as we have made indicated that the extent of this contamination (with protein other than gliadin)[5] cannot exceed 0.6 per cent of the entire food mixture, a quantity of "normal protein" far too small as, we have convinced ourselves by other studies directed to this point, to meet the nutritive deficiency of gliadin in respect to growth (26).

All ordinary milk is infected with bacteria during the process of milking, and it is well known that milk contains proteolytic ferments which may well within a few hours convert a significant amount of the protein of the milk into cleavage products sufficiently simple to escape precipitation by the protein precipitants. Milk is also known to contain all the constituents of the blood in small amounts and modern re-

[4] Italics, ours.

[5] Words in parentheses, ours.

searches of Folin and Denis (34) and Van Slyke and Meyer (35) have demonstrated that the blood is a dilute solution of amino-acids. All things considered we believe that the assumption is unwarranted that "protein-free milk" may not serve as a very significant source of various amino-acids. In the experiments described the assumption was made that the lactating mother was constructing from gliadin, supposed at that time to be practically free from lysine, the milk proteins which contain an abundance of this cleavage product of proteins. Accordingly, the assumption that growth of the young which were nursing the mother on this relatively lysine-poor diet affords proof of the synthesis of lysine or a difference between the chemical requirements of an animal for growth as contrasted with maintenance, cannot be regarded as resting upon a sound experimental basis.

The considerations which have been discussed in their relation to the validity of the proof of the ability of the mammary gland to produce syntheses not possible for the other tissues of the body, and the theory that the processes of maintenance differ chemically from those of growth, serve likewise to emphasize further the fact that feeding experiments in which "protein-free milk" is used cannot be regarded as in any way comparable with experiments in which the diets consist of carefully purified food substances, together with butter fat as a source of fat-soluble A and suitably prepared extracts of natural foods to furnish the second unidentified dietary essential, water-soluble B. Only with diets of this type can the individual proteins be compared in a way which reveals their relative biological values. "Protein-free milk" contains a liberal amount of water-soluble B, and a small and inadequate amount of fat-soluble A (31). Much of the work done with diets containing "protein-free milk" may possibly have led to correct conclusions, but it is impossible to tell which of the results are trustworthy and which are not until the work is repeated with suitably controlled diets.

Hart and Humphrey (36) have reported the results of several experiments so planned as to compare the efficiency of a number of protein mixtures for transformation into milk. These show decided differences in the biological values of proteins for milk production, entirely analogous to what is well established with respect to difference in the values of individual proteins for growth (37).

As yet it has not been shown that the mammary gland is any more efficient in the utilization of food protein for milk production than are the tissues of the young of a rapidly growing species for the construction

of new body tissue. Apparently the same limitations hold in both cases.

The quantitative comparison of the ability of the young to utilize food proteins for growth as compared with the ability of the mammary gland to utilize them for the synthesis of milk proteins, is attended with peculiar difficulties. The rate at which the young can be expected to utilize protein for storage as new body tissue will be determined by the "growth impulse" of the species (37). The human infant is not capable of multiplying its initial weight by much more than 3 during the first year of life and cannot accordingly be expected to retain a high percentage of the protein taken as food for the formation of body tissue. Its growth impulse is low. The domestic pig, on the other hand, has the greatest growth impulse of any species with which we are familiar. It is capable, when its diet is highly satisfactory, of multiplying its initial weight by about 200 during the first year of life. The pig is capable of retaining protein taken in the food for the formation of body tissue, at a much greater rate than is any species whose capacity for growth is decidedly less than its own. It is only by making use of the most rapidly growing species of which we know that we can expect to secure data as to the actual biological values of a series of proteins. Only the most rapidly growing species can be expected to retain food protein at the maximum rate made possible by the peculiar relationships among the amino-acids which it yields on digestion.

A similar difficulty is met with in attempting to determine the value of the food proteins for the formation of milk proteins. The extent to which the lactating animal is capable of converting food into milk depends not alone upon the quantity of the food, her capacity to digest and assimilate food and on the quality of the food proteins, etc., but likewise in no small degree upon the inherited milk-producing tendency of the individual. The results of the extensive testing of individual cows by the Babcock fat test during the last twenty-five years, have revealed the fact that in most dairy herds there are some cows which are not capable of profitable production, even when given the best food and care. Milk-producing capacity is an inherited trait and it is only in animals with the power of lactation highly developed that one may expect to observe utilization of food proteins for transformation into milk proteins at the maximum rate at which the quality of the food proteins, depending upon their yields of the essential amino-acids, will permit.

Variability in the composition of the milk depending upon the composition of the mother's food. A number of investigations have been directed toward the study of the variability in the content and relationships among the inorganic elements of the milk as influenced by the character of the food. Von Wendt (38) found that the extent of the variability of any of the constituents of the milk is very slight, too slight indeed to be considered as of significance as regards the nutritive value of the secretion. In his experiments cows were fed mixed rations of fairly satisfactory quality for milk production. To these he added certain salts and studied the possibility of *increasing* the output of any element in milk. It is not surprising that he was unable to do this since the kidneys and intestine aid in preventing an accumulation of any mineral elements in the blood.

G. Von Bunge states that as early as 1872 he called attention to the question as to whether the mammary gland secretes inorganic constituents in the same relationship as they occur in the ash of the suckling (39). He called attention to the fact that the blood has an entirely different inorganic content from that of the milk which is secreted, although the mammary gland extracts the inorganic materials from the blood stream which flows through it. The following table has become a classic as an illustration of the relationship between the character of the inorganic content of milk from different species of animals and the content of the same elements in the young animal.

100 PARTS OF ASH CONTAIN	NURSING YOUNG ANIMAL			DOG MILK	DOG BLOOD	DOG BLOOD SERUM
	Rabbit	Dog	Cat			
K_2O	10.8	8.5	10.1	10.7	3.1	2.4
Na_2O	6.0	8.2	8.3	6.1	45.6	52.1
CaO	35.0	35.8	34.1	34.4	0.9	2.1
MgO	2.2	1.6	1.5	1.5	0.4	0.5
Fe_2O_3	0.23	0.34	0.24	0.14	9.4	0.12
P_2O_5	41.9	38.8	40.2	37.5	13.2	5.9
Cl	4.9	7.3	7.1	12.4	35.6	47.6

Bunge pointed out that the fact that the potassium content of milk ash is somewhat greater than in the ash of the suckling is to be accounted for on teleological grounds. The growing animal is relatively richer in potassium than in sodium. This depends on the increase in the content of the potassium-rich muscle and the relative decrease in the proportion of sodium-rich cartilage.

The great and constant differences in the ash of the blood and milk are sufficient to dispose of the argument that the secreting gland acts through the principle of filtration, removing the proteins, etc., from the blood.

Babcock (40) has described experiments with cows which he deprived of salt (NaCl) during lactation, and his results are of special interest in relation to the interpretation of our data. He deprived cows of sodium chloride, other than that contained in their food, for varying periods up to fifteen months and noted no decrease in the yields of milk up to a short time before the animals began to fail rapidly. Some actually died from sodium chloride starvation and others were saved from death by the administration of salt. The fat content of the milk of cows receiving an inadequate salt supply was slightly higher than in milk from the control group.

Interesting observations showing the influence of over-feeding of sodium chloride on the character of the milk of cows are also cited by Professor Babcock (40). Lowe in 1861 found 13 per cent of solids in the milk of a cow receiving 2.5 ounces of salt per day. She was given double this amount for three days and the milk on the fourth day contained but 8 per cent of solids. After this the cow was given the usual 2.5 ounces of salt per day and the milk gradually returned to its normal composition. Mendel (41) reports an experiment in Switzerland which showed marked decrease in the solids of the milk as the result of feeding high intakes of salt to dairy cows. This effect is, of course, due to an additional excretion of salt in the milk and with an excessive output of water, which dilutes the milk.

The effects of under-feeding and of faulty diets on the persistence of milk secretion in the lactating female. Eckles and Palmer (42) have conducted very thorough and painstaking experiments on the influence of under-feeding on the flow and composition of the milk in cows. Their results reveal the fact that in the early part of the lactation period, cows are able to remain stationary in milk flow and produce the normal amount for forty days with only 60 to 75 per cent enough energy after subtracting the maintenance requirement. They observed a marked difference in the effect of a subnormal plane of nutrition on the milk flow depending on the part of the lactation period in which the experiment was carried out. During the later portion of the lactation period there is a reduction of the flow in response to under-feeding while this, as stated above, is not the case when the under-feeding follows closely upon parturition. Eckles and Palmer explain this dif-

ference on the assumption that milk production is caused by both a chemical stimulus and a nervous stimulus. The former of these is principally responsible for the activity of the mammary gland during early lactation and decreases in its intensity as the lactation progresses, and is finally replaced in great measure by the nervous stimulus. So long as the chemical factor is chiefly the regulating factor governing the gland, milk production is in great measure independent of the plane of nutrition of the cow.

If this law holds good generally for lactating animals, the assumption would be valid that in all the nursing experiments reported in this paper in which mother rats were suckling young while taking diets on which the young themselves were not able to grow after the weaning period, the mothers were producing approximately the normal amount of milk.

In the charts are shown records in which young rats grew at half normal rate on the milk of mothers whose food was of such a nature that it could not produce growth directly. We have the analogy with the cow as evidence that they were producing approximately the normal amount of milk, and it would seem to be the only logical conclusion that the young failed to grow at the full normal rate because of the poor quality of the milk produced.

If then, as seems to be demonstrated by the available data, the mammary gland has no special synthetic power in the synthesis of amino-acids and is limited in the same way as is the young to a supply of these in the food, and as we have previously shown, the dietary essentials fat-soluble A and water-soluble B are not found in the milk unless they are in the food of the mother (43), it becomes of special interest to discover in just what manner the mother acts in a protective capacity toward her young when her own diet is itself inadequate for the nutrition of the young.

The effect produced in the young rats suckled by mothers whose food supply was limited to a single grain, as wheat, maize or oat kernel, with or without single or multiple additions of purified food substances was, it seems certain, the result of the poor quality of the milk rather than of a limitation in the supply. A fact not hitherto appreciated if indeed at all suspected, viz., that the quality of milk as judged by its growth-promoting power is dependent in a surprising degree upon the quality of the diet of the mother, is shown in a conclusive manner. The differences in quality, if we can judge by analogy with the cow, are of such a nature as not to be detectable by any of the ordinary methods

of chemical analysis. Even extraordinary methods of chemical analysis could reveal the deficiencies of such milk only with respect to its inorganic content.

We have sought in the experiments reported in this paper to analyze by the biological methods, which we have made use of in the study of the problems involved in the nutrition of the growing young, the peculiar rôle of the mother in safeguarding the young in early life.

These experiments are not directly comparable with those of Eckles and Palmer in certain important respects. They fed mixtures of leafy foods and grains, mixtures which we have shown to be dietetically complete (44) in that all the essential dietary factors were present and in no very great degree were there unsatisfactory proportions among the several factors. Their experiments involved only limitations as to amount. Our experimental conditions reported in this paper imposed special features—dietary problems not met with in the case of the herbivora but common enough among human beings who do not make use of dairy products as foods, unless they partake of the leafy vegetables in much greater amounts than are commonly eaten in this country. Eckles and Palmer were feeding a diet which would induce growth in a young animal if supplied in sufficient amount. The seeds which formed the basis of our food mixtures would not support growth because of three types of deficiencies, viz., inorganic content, protein and fat-soluble A. With such diets the distinct limitations of the maternal organism in the matter of secreting normal milk are easily seen.

The results reported with cows are in harmony with those reported by Decaisne (45) who observed that during the siege of Paris in 1870, young and vigorous women maintained without loss of weight and in some cases induced gains in their infants, when they were themselves fasting or nearly so.

The studies here reported (charts 1 to 6) show that the lactating mother is limited in a general way in all respects as are the growing young with respect to her ability to effect chemical transformations of one food complex into another, utilizing food proteins for milk production only to the extent that they yield amino-acids in proportions suitable for rearrangement into milk proteins. She is, however, a very important factor of safety for her young in that her mammary tissues can remove from the blood all elements necessary for the production of milk, approximating more nearly the normal in quality than was the food from which it was produced. She can pass these on into the milk in decidedly more favorable relationships than they exist in her food.

This the mammary gland can do when nourished by blood which contains certain inorganic elements in such relationships as render the circulatory fluids of the body a pabulum from which the tissues of the young cannot secure satisfactory supplies to permit the cells to grow, even though the organic portion of the diet is satisfactory. In other words she can present to her dependent offspring a more satisfactory inorganic food supply than she herself receives, and through the medium of the mother the young is enabled to progress well toward the attainment of the adult size in an environment in which the food supply is of such character as would not support growth if it were not for the medium of the mother. (See charts 3 to 5.)

The well established facts concerning the relation between the character of the diet and the formation of milk may be briefly summarized as follows:

1. The evidence seems conclusive that there is no special synthetic power of the mammary tissue by reason of which amino-acids can be formed which are not found in the food. In other words, the maternal organism is limited in milk secretion in the same way as is the growing animal in regard to its amino-acid supply. Both must have the same list of amino-acids preformed and circulating in the body fluids. Diets employed in this type of study must be rigidly controlled so as to preclude the possibility of the animals procuring the special complexes from "protein-free milk," feces, etc., on whose absence the validity of the observations rest.

2. The mammary gland performs the function of elaborating proteins of extraordinary biological value from the products of digestion of the food proteins. This involves no synthetic activity by which one amino-acid is transformed into another but only a selective absorption from the blood and reconstitution of the amino-acids which circulate in the blood into complexes, the milk proteins, which in turn are, when taken as food, very efficiently transformed into tissue proteins for growth.

3. The mammary gland picks up from the blood both the chemically unidentified food essentials, fat-soluble A and water-soluble B, and passes these into the milk, but it is unable to produce either of these substances anew. When one or the other of them is absent from the mother's diet, it is not found in the milk (43). We have shown the possibility of producing milk, poor or lacking in each of these substances and therefore not capable of inducing growth.

4. The mammary gland has the capacity to function approximately

normally when nourished by a blood stream which contains the mineral elements, sodium, chlorine and calcium in amounts too low to enable the tissue cells to grow. This is shown by the fact that the inorganic content of the milk produced from a diet whose content of these elements is too low to admit of growth in the young is of distinctly better quality for supporting growth than is the food from which it was formed. This represents a very important factor of safety in the preservation of the species.

5. It is evident from the character of the curves of growth of the young in charts 1 to 6 that the quality as well as the quantity of the proteïn of the diet, together with the content and character of the inorganic portion of the food supply, are factors of importance for milk production commensurate with that of an adequate amount of both the fat-soluble A and water-soluble B.

6. Even after the young has attained a stage of development at which it can eat of the mother's ration, the supplementary contribution of milk by the mother serves to greatly enhance the proteins derived from vegetable foods and to partially correct the inorganic faults in the latter as well as to give the young a much greater supply of fat-soluble A than it could itself ingest in the form of vegetable foods. Fat-soluble A is not abundant in the vegetable foods and the action especially of the herbivorous mother in concentrating in the milk fats the intake of this substance in her large intake of food, constitutes a surprising protective relationship of the mother to her young. The results of the present series of experiments show that milk is superior to the vegetable mixtures on which the herbivorous mother feeds in its physical properties, its easy digestibility and in its favorable inorganic content.

The experiments described in the charts demonstrate the following facts: (1) That the mother may take a single seed as food and produce milk in sufficient quantity and of such quality that growth can proceed at a subnormal rate whereas the young could not themselves grow at all on the mother's diet; (2) when the inorganic deficiencies of the seed are corrected, the mother can induce much better growth in the young than without such correction; (3) *the improvement of the protein or the fat-soluble A content of the seed causes little improvement in the quality of the milk when the mineral content of the diet remains uncorrected.*

These results for milk production are in agreement, except in degree, with the behavior of young when fed a seed supplemented in the same manner. The mother can take a seed as her sole food and put into the milk an inorganic salt content which is very much more satisfactory for

the nutrition of the young than is that contained in the seed. It is in this respect that she serves as the greatest factor of safety in the nutrition of her young. The other ways in which the maternal organism assists the relatively helpless young in its nutrition have been already pointed out.

It is now well established that the seeds, tubers and root foods are closely comparable from the dietary standpoint in that their deficiencies are of the same type and order, all being storage organs relatively poor in cellular elements. Muscle tissue is principally composed of highly specialized contractile tissue and contains but relatively little substance comparable in its metabolic activity with the cellular organs such as the liver, kidney and other glands. It should, according to our theory of the relation of dietary properties to function, be classed with the storage organs of plants rather than with cell-rich tissues such as the leaf. It is not possible to make up diets derived from even these four types of foodstuffs (seeds, tubers, roots and muscle tissues (17)) in any combinations which will induce normal nutrition. It should follow from the studies now reported, and there can be little doubt of the correctness of this view, that milk produced by a mother whose food consists entirely of seed products, tubers and meat, will not be of very satisfactory quality for inducing growth.

DISCUSSION OF RESULTS

We have, in previous papers, shown that there is but one way in which an adequate diet can be made up entirely from vegetable sources, provided we ignore the possibility of the chance supply through the drinking water of such a mineral element addition to the diet as will make good the deficiencies of all the seeds in calcium, sodium and chlorine, viz., by making suitable combinations of the leaf and seed (44). There is small chance that a diet derived entirely of seeds will, even when the water furnished contains the proper minerals, prove of very satisfactory quality for the support of growth. We have emphasized the fact that the present tendency in parts of the United States to derive the diet almost entirely from seeds, tubers and meat, is responsible for the lowered vitality, loss of efficiency and the appearance of diseases of dietary origin (46). Our studies also afford abundant proof that highly successful diets can be made up of mixtures of seeds and milk (17). The importance of the dairy industry as a factor of

safety in the nutrition of the adult population of the more progressive nations of the world makes the results of these studies of the relation of the mother to the young applicable to the every day problems in the nutrition of man, adult as well as infant.

The three most important seeds employed as foods in America are the wheat, oat and maize kernels. Each of these contains all the elements and chemical complexes which are essential in the diet of the growing young animals, yet each when it serves as the sole source of nutriment for a young animal fails to induce any growth and cannot long maintain life. The most elaborate chemical analysis throws practically no light on the reasons for the failure of nutrition in animals so fed. Even mixtures of these three seeds, in any proportions, do not support growth. The reasons for this we have shown by our biological method for the analysis of foodstuffs (22) to rest in the inadequate character of the inorganic moiety of the several grains as the first limiting factor, the shortage of the fat-soluble A as the second, and the relatively poor quality of the proteins as the third factor. The latter fault is less pronounced in mixtures of the grains than in each of them as the sole source of protein.

Among peoples who subsist almost entirely upon polished rice and fish, the disease beriberi is of common occurrence and it not infrequently happens that infants nursing mothers suffering from the disease likewise contract beriberi in the first months of life. The cause of beriberi is now well known to be the consumption of a diet which may be unsatisfactory in several respects, but the specific cause of the syndrome is the selective fasting of the individual for the unidentified dietary essential water-soluble B (4). Diets so made up that they are known to be adequate in every respect, except for the shortage of this substance, induce the onset of the disease within a few weeks. Andrews (47) attempted to throw light on the etiology of infantile beriberi by inducing Philippine women, whose infants had just died of beriberi, to nurse young pups. These in all cases developed the paralysis of the posterior extremities which is one of the characteristic symptoms of the disease. The cause of the disease had not at that time been established, whether it is due to some toxic agent or to a lack of some essential substance in the diet. Andrews' experiments are of great interest in connection with our own, in which we made up diets which were deficient only in respect to the dietary factor, water-soluble B, and observed that young rats were unable to grow on the milk of mothers after the latter were restricted for a few days to these food

mixtures, but responded with growth in about forty-eight hours after the addition of an alcoholic extract of wheat germ, which supplied the missing dietary essential (43). Andrews' studies with human milk are confirmed and explained by our own with rats, and establish the fact *that milk production may take place under dietary conditions such that the product is deficient in certain complexes necessary for growth.*

We have recently called attention to the fact that there is a second dietary deficiency disease analogous with beriberi, in that it is the specific result of a shortage of the chemical substance fat-soluble A in the diet. This is a type of xerophthalmia of dietary origin and is characterized by an edematous condition of the eyes, followed speedily by blindness unless the missing dietary essential is supplied (5). Clinical evidence is available in the observations of Mori (48), Bloch (49), H. Rønne (50), Czerny and Keller (51), that this syndrome which became known through nutrition studies on animals, has repeatedly occurred as a human disease.

As we have elsewhere pointed out (5), there is but one way to produce this condition of the eyes, viz., by selectively fasting an animal for the dietary essential fat-soluble A, and it can be relieved only by supplying in the diet the missing dietary complex. From among the numerous citations in the literature of eye affections among poorly nourished peoples in many parts of the world, we have found it impossible to decide whether the xerophthalmia of dietary origin has been sometimes confused with trachoma or other eye infections. It will be of special interest if clinicians will keep in mind the possibility that edema of the tissue surrounding the eyes, inflammation of the cornea with or without perforation, followed in extreme cases by blindness, may have their origin in a specific lack in the diet in a manner entirely analogous to beriberi. The subject is mentioned here because milk may be very poor in the dietary essential, fat soluble A, when the diet of the mother is derived almost wholly from seeds and seed products, especially those derived from the endosperm, e.g., wheat flour, degerminated corn meal, polished rice, starch, sugars, syrups, together with vegetable fats or the reserve fat deposits of the animal body (lard, suet, etc.).

The light which the experimental data described in this paper throws upon the relation between the quality of the diet and the quality of the milk as a food, may be considered in its possible relation to the high incidence of rickets among infants in classes of people who live in poverty. Among the very poor there is a strong tendency to restrict the

diet to bread and other seed products, syrups and molasses, together with potatoes, sweet potatoes and small amounts of meat. We now know that such diets do not produce satisfactory results in the nutrition of animals, and that the milk of the mother taking such diets is likely to be of very poor quality as food for her infant. It seems not improbable that future investigations may show a relationship between the lowered resistance of infants so fed and the development of rickets, whatever may be the exact nature of the immediate causative agent of the disease. Hess (52) has recently described the diets of negro mothers in the Columbus Hill District in New York City, whose infants almost universally suffer from rickets. The mothers' diets consist of seed products, tubers and meat almost exclusively. Milk, the leafy vegetables and fruits are scarcely eaten at all.

One of the most interesting and surprising facts which has come from our systematic studies with simplified diets is the remarkable dependence of the young animal upon a suitable content of several mineral elements in its food supply. An animal cannot grow on one of the cereal grains as the sole food supply but if 1 per cent of sodium chloride and 1.5 per cent of calcium carbonate be added to a cereal grain a considerable amount of growth may take place. All the elements contained in these salts are present in the seeds but not in sufficient amounts. Apparently the kidney fails to hold back these elements as effectively as it should and thereby renders the young animal much more in danger of failing to secure a sufficient intake of these elements than would be the case if the kidney were able to excrete the waste products of metabolism and at the same time hold back and permit to accumulate in the blood stream and lymph a concentration of mineral elements which would be favorable for the nutrition of the body tissues during growth. This it fails to do, and growth can take place in those mammals which have been studied only when certain mineral elements are coming from the alimentary tract at or above a certain rate. The kidney seems, therefore, in some cases involving a low intake of inorganic salts in the diet, to be the tissue whose limitations determine the ability of the young animal to grow.

A suggestive feature of the results of these studies is the deduction which may reasonably be drawn concerning the quality of the blood as a pabulum for the nourishment of the tissues in general when the diet is derived too exclusively from the seeds of plants. It has been pointed out how different is the inorganic content of the milk as compared with that of the blood serum, and it is well known that the proteins of

milk are the specific product of the mammary tissue and are not found in the blood.

The lactose is derived from the glucose which is ever present in the blood. These facts seem to indicate the inability of the mammary glands to function properly because of the poor quality of the blood in respect to salts or protein cleavage products, or to the fat-soluble A or water-soluble B, when the blood gets its supply of these substances from an intestine which is digesting cereal grains exclusively. As stated above, the product of the mammary gland is specific and widely different from the blood, yet it is sensitive to such changes in the blood as may arise from the too exclusive eating of seeds. The question which suggests itself is, how do the remaining tissues of the body fare when purveyed to by a blood which does not admit of the production of growth-promoting milk? Diets derived almost exclusively from seeds such as peas, navy beans, corn, wheat, rice and rolled oats and tubers, such as potato, cause stunting and admit the early onset of signs of senility, and the span of life is short (17). The debilitating influence of such diets is a factor in the public health which should be more generally appreciated than it is both by the physician and the laity. It requires grave dietary faults to produce such serious conditions as scurvy, rickets and beriberi. There are tens of thousands of human mothers who are attempting to nurse infants on diets derived too largely from the seeds of plants and their milled products. Such diets produce inferior milks and may be the cause of grave disorders in the very young. Dietary mistakes of a minor character which debilitate the tissues and lead to inefficiency, lack of resistance to bacterial infection, and pave the way to the onset of errors in metabolism are very common. In but few instances does the human machine run past middle life without developing a rattle somewhere. It is clear from experimental evidence now available that there are causes other than those proposed by Metchnikoff responsible for the gradual diminution of vitality which daily brings us nearer to the condition where we are classed with the great army of the unfit.

It is certain that milk is a better food for the young during the suckling period that is any ration which can possibly be compounded from vegetable sources. If the mother is practically limited in respect to the synthetic capacity of her mammary tissue in the same way that the suckling is, except in the formation of milk sugar from glucose, is it only in the physical properties and digestibility of milk and in the peculiar character of its carbohydrate that milk is more suited for the

nutrition of the suckling than is the coarser food eaten by the mother? The experiments described in this paper indicate that a shortage of certain inorganic salts in the diet of the lactating mother may become a factor of great importance in determining the quality of the milk. (Compare charts 3, 4, 5 and 6.)

It may be argued that the faulty diets employed in our experiments (charts 1 to 6) caused depression of the growth of the young by reducing the milk production of the mothers rather than by changing the composition of the milk with respect to some one or more of the important constituents. We cannot state from actual records of milk production in the rat whether the volume of milk produced remained sufficient for the growth of the young throughout the periods of depressed growth, but the observations on women (45) and cows (42) just cited bear directly on this point. Furthermore, in our own records young are regularly shown to grow long after they become able to eat of the mother's diet, when the latter is of a character which cannot support growth when it serves as the sole food supply. There can be no other explanation for this than that the mothers continue to supplement the food supply of the young with fairly liberal contributions of milk.

From the experiments just cited we have data strictly comparable to our own where mother rats are limited for their supply of mineral elements to the single seed which furnished the major portion of the diet. The seeds, wheat, oats and maize, we have shown are too poor in sodium, chlorine and calcium to permit young animals to grow appreciably, and we see in the case of the lactating mother attempting to induce growth in a litter of four young an inability to produce milk of a character which enables the young to grow beyond a limited rate, but the milk is better for the nutrition of the young than is the mother's diet. From the data furnished by Babcock (40), by Eckles and Palmer (42) and by Decaisne (45), as well as by our own charts, it seems certain that failure to produce a sufficient quantity of milk was not the cause of partial stunting of the young, but rather too low a content of those mineral elements which the seeds fail to supply in amounts sufficient to meet the needs of a growing animal. These experiments seem to afford evidence that diets containing no more of certain inorganic elements than are furnished by the more important seeds employed as human and animal foods may lead to the production of milk of poor quality as a growth-promoting food.

TABLE 1

Rolled Oats

NUMBER OF EXPERIMENT	RATION	WEIGHT AND APPEARANCE AT 14 DAYS	AGE AT WHICH YOUNG BEGAN TO DIE	WEIGHT AND APPEARANCE AT 28 DAYS	WEIGHT AND APPEARANCE AT ABOUT 50 DAYS	
Chart 1						
1978	Rolled oat 60.0; NaCl 1.0; $CaCO_3$ 1.5; dextrin 32.5; butter fat 5.0	4 young weighed 92 grams and were in excellent condition	First young died at 57 days of age	4 young weighed 163 grams, and were very alert	4 young weighed 225 grams and were in good condition (very active)	Good
	A second group duplicated	these results very closely				
843	Rolled oats 95.0; butter fat 5.0	4 young weighed 84 grams and were in excellent condition	First young died at 60 days of age	4 young weighed 127 grams and were in excellent condition (very active)	4 young weighed 148 grams	Exce
	A second group duplicated	these results very closely				
899	Rolled oats 95.3; salts 4.7	4 young weighed 101 grams and were in excellent condition	First young died at 52 days of age	4 young weighed 139 grams and were in excellent condition	4 young weighed 190 grams and were in good condition	Good
	A second group duplicated	these results very closely				
983	Rolled oats 77.0; casein 18.0; butter fat 5.0	4 young weighed 75 grams, were very small but active	First young died at 48 days of age	4 young weighed 100 grams and were small and active	3 young weighed 111 grams and were small and inactive	Loss ba
	A second group duplicated	these results very closely				
738	Rolled oats 100.0	4 young weighed 70 grams, small but in good condition	First young died at 37 days of age	4 young weighed 91 grams	4 young at 40 days weighed 102 grams	
	A second group duplicated	these results very closely				
Chart 2						
980	Rolled oats 80.0; skim milk powder 20.0	4 young weighed 77 grams and were in excellent condition		4 young weighed 159 grams and were in excellent condition		
	A second group duplicated	these results very closely				
1019	Rolled oats 77.3; casein 18.0; salts 4.7	4 young weighed 99 grams and were in excellent condition (very active)		4 young weighed 218 grams and were very sleek	4 young at 41 days weighed 243 grams and were in good condition	
	A second group duplicated	these results very closely				
1020	Rolled oats 85.3; gelatin 10.0; salts 4.7	4 young weighed 81 grams, but were small and inactive		At 25 days the 4 young weighed 157 grams and were in good condition		
	A second group duplicated	these results very closely				
984	Rolled oats 85.0; gelatin 10.0; butter fat 5.0	4 young weighed 71 grams, were small but active		4 young weighed 115 grams and were active	4 young weighed 214 grams, good condition (alert)	Sick
	A second group duplicated	these results very closely				
949	Rolled oats 90.0; gelatin 10.0	4 young weighed 66 grams, but rather cold and emaciated		4 young weighed 112 grams, small and active. Their eyes were swollen	4 young weighed 148 grams	Ver
	A second group duplicated	these results very closely				
948	Rolled oats 82.0; casein 18.0	4 young weighed 52 grams, very emaciated	Young were all dead at 23d day. This is the longest time that any of them remained alive on this mixture. Some died at 3 days, 7, 10, etc.			Goo

APPEARANCE DAYS	WEIGHT AND APPEARANCE AT ABOUT 50 DAYS	CONDITION OF MOTHER RAT	REMARKS
d 163 grams, and rt	4 young weighed 225 grams and were in good condition (very active)	Good but nervous	At about 53 days the hair of the young was short. They presented a very poor appearance
d 127 grams and llent condition)	4 young weighed 148 grams	Excellent	On the 52d day the eyes of the young were inflamed
d 139 grams and llent condition	4 young weighed 190 grams and were in good condition	Good	On the 53d day the young died. There were no signs of soreness
d 100 grams and nd active	3 young weighed 111 grams and were small and inactive	Loss of hair on sides, sores on back but not tail or ears	A few of the young at 51 days tottered while walking and were very immature
ed 91 grams	4 young at 40 days weighed 102 grams		On about the 20th day a few of the young would throw themselves about the cage and scream during this performance. Several of the young went into coma and died
ed 159 grams and llent condition			
ed 218 grams and leek	4 young at 41 days weighed 243 grams and were in good condition		
4 young weighed nd were in good			
ed 115 grams and	4 young weighed 214 grams, good condition (alert)	Sick	
ghed 112 grams, active. Their rollen	4 young weighed 148 grams	Very nervous. Hair shaggy	The young were very small and puny. Their eyes were swollen and abdomens distended
		Good	Seven females were fed this mixture and their young all died at a very early date. The intestines were filled with gas. We are unable to account for the high mortality of these young

TABLE 2

Wheat

NUMBER OF EXPERIMENT	RATION	WEIGHT AND APPEARANCE AT 14 DAYS	AGE AT WHICH YOUNG BEGAN TO DIE	WEIGHT AND APPEARANCE AT 28 DAYS	WEIGHT AND APPEARANCE AT ABOUT 50 DAYS	
Chart 3						
842	Wheat 95.0; butter fat 5.0	4 young weighed 91 grams and were in excellent condition	First young died at 52 days of age	4 young weighed 142 grams and were in good condition	3 young weighed 139 grams	Very s sore
	A second group duplicated	these results very closely				
709	Wheat 100.0	4 young weighed 77 grams. They were small but in good condition	First young died at 50 days of age	4 young weighed 117 grams. They were small but in good condition	3 young weighed 106 grams	Very
	A second group duplicated	these results very closely				
946	Wheat 86.6; casein 13.4	4 young weighed 65 grams. They were small and in poor condition	First young died at 37 days of age	4 young weighed 85 grams. They were small but active	3 young weighed 105 grams. They were very nervous	Good
	A second group duplicated	these results very closely				
Chart 4						
1018	Wheat 81.8; casein 13.4; salts 4.8	4 young weighed 101 grams		4 young weighed 220 grams. In excellent condition	4 young at 39 days weighed 385 grams	
	A second group duplicated	these results very closely				
1943	Wheat 92.5; NaCl 1.0; $CaCO_3$ 1.5; butter fat 5.0	4 young weighed 70 grams. In excellent condition (very alert)		4 young weighed 125 grams. Very alert	4 young weighed 209 grams. In good condition	Good
	A second group duplicated	these results very closely				
982	Wheat 81.6; casein 18.0; butter fat 5.0	4 young weighed 93 grams. In very good condition		4 young weighed 140 grams. Very good condition	4 young weighed 183 grams	Very con
	A second group duplicated	these results very closely				
900	Wheat 95.2; salts 4.8	4 young weighed 80 grams. In good condition (active)	First young died at 67 days of age	4 young weighed 132 grams. Good condition	4 young weighed 166 grams	Hair

* We have elsewhere described the relation of abnormal skin and eye conditions to faulty diet (22).

ND APPEARANCE 28 DAYS	WEIGHT AND APPEARANCE AT ABOUT 50 DAYS	CONDITION OF MOTHER RAT	REMARKS
hed 142 grams and od condition	3 young weighed 139 grams	Very emaciated, but no signs of soreness	The young were very inactive toward the end of the experiment but there were no signs of sore ears, tails or eyes*
ighed 117 grams. small but in good	3 young weighed 106 grams	Very emaciated	The eyes of the young and the mother were swollen and inflamed. Two of the young had sore ears
ighed 85 grams. small but active	3 young weighed 105 grams. They were very nervous	Good	There were no sore eyes but the young were at all times small for their age
ighed 220 grams. t condition	4 young at 39 days weighed 385 grams		
ighed 125 grams.	4 young weighed 209 grams. In good condition	Good	These four young at 85 days of age weighed 385 grams. They were very sleek; no signs of soreness
ighed 140 grams. condition	4 young weighed 183 grams	Very emaciated and in poor condition	The four young at 63 days weighed 225 grams. They were alert but shaggy
ighed 132 grams. ition	4 young weighed 166 grams	Hair thin on sides	The young were quite bald and their eyes were swollen

TABLE 3

Corn

NUMBER OF EXPERIMENT	RATION	WEIGHT AND APPEARANCE AT 14 DAYS	AGE AT WHICH YOUNG BEGAN TO DIE	WEIGHT AND APPEARANCE AT 28 DAYS	WEIGHT AND APPEARANCE AT ABOUT 50 DAYS	C
Chart 5						
898	Corn 96.3; salts 3.7	4 young weighed 66 grams and were small but very active	First young died at 125 days of age	4 young weighed 105 grams and were in good condition	4 young weighed 134 grams	Very
	A second group duplicated	these results very closely				
737	Corn 100.0	4 young weighed 71 grams; one eye of one of the little rats was swollen	First young died at 45 days of age	4 young weighed 106 grams and were in fair condition	4 young weighed 83 grams	Emac
	A second group duplicated	these results very closely				
947	Corn 82.0; casein 18.0	4 young weighed 72 grams, small, but very alert		4 young weighed 105 grams, very small but active	4 young weighed 145 grams	Look
	A second group duplicated	these results very closely				
Chart 6						
1017	Corn 78.3; casein 18.0; salts 3.7	4 young weighed 113 grams and were in excellent condition		4 young weighed 178 grams	4 young at 47 days weighed 320 grams	Grow
	A second group duplicated	these results very closely				
844	Corn 95.0; butter fat 5.0	4 young weighed 75 grams and were small and inactive	First young died at 77 days of age	4 young weighed 88 grams and were small and inactive	4 young weighed 110 grams	Good
	A second group duplicated	these results very closely				
	First period					
981	Corn 77.0 casein 18.0; butter fat 5 0	4 young weighed 98 grams and were small and active		4 young weighed 128 grams	4 young weighed 186 grams and were in good condition but rather small	Lost
	Second period					
	NaCl 1.0; $CaCO_3$ 1.5 replaced part of the corn					
	A second group duplicated	these results very closely				
	First period					
1942	Corn 92.5 NaCl 1.0; $CaCO_3$ 1.5; butter fat 5.0	4 young weighed 62 grams. Not very active		4 young weighed 86 grams and were very small and babyish	4 young at 44 days weighed 128 grams. The protein addition was made at this time	Emac
	Second period					
	Casein 18 0; replaced part of the corn					
	A second group duplicated	these results very closely				

ND APPEARANCE 28 DAYS	WEIGHT AND APPEARANCE AT ABOUT 50 DAYS	CONDITION OF MOTHER RAT	REMARKS
hed 105 grams and od condition	4 young weighed 134 grams	Very poor	On the 19th day one of the little rats had one eye swollen. At the end of 136 days there were 3 young left. These were in very poor condition the hair was thin and the eyes were swollen but no sore ears or tails
hed 106 grams and r condition	4 young weighed 83 grams	Emaciated	The eyes of one of the young were swollen and shut on the 19th day. On the 30th day the eyes were all right but later the eyes were swollen again. The young were very inactive toward the end of the experiment.
ighed 105 grams, but active	4 young weighed 145 grams	Looked old	The young were very nervous and died at 63 days of age
ghed 178 grams	4 young at 47 days weighed 320 grams	Growth on nose	
ghed 88 grams and and inactive	4 young weighed 110 grams	Good	Eyes of the young were swollen but not inflamed. Some of the young were bald in spots
ghed 128 grams	4 young weighed 186 grams and were in good condition but rather small	Loss of hair on sides	At 66 days of age, or 15 days after the addition of NaCl and $CaCO_3$, the 4 young weighed 308 grams and were in excellent condition. The young rats were growing a new coat of hair
ghed 86 grams and small and babyish	4 young at 44 days weighed 128 grams. The protein addition was made at this time	Emaciated but no soreness	Twenty-eight days after protein addition, the 4 young weighed 337 grams and were getting a new coat of hair

EXPERIMENTAL METHODS

In order to study the extent to which the mother can serve as a protective agent in producing milk of a character suitable for the nutrition of her young when she is fed upon a diet on which the young, after weaning, are not able to grow, we adopted the following procedure: The female rats were fed our 211[6] ration until they delivered their young. The litter was then in all cases reduced to four young in order that the nutritive undertaking of all the mothers should be comparable and in no case burdensome. The mother was then restricted to the diet described in the chart with her curve, and frequent weighings were made of both mother and young, from which the curves were plotted. The curve of the mother is placed at the bottom of the chart and the curve showing the collective weighings of the four young is placed directly above. A drop in the curve of the young indicates that at that point the litter was reduced by the death of one or more, to three, two, etc., young. Supplementary data regarding the condition of the young and mothers are presented in tabular form in tables 1, 2, and 3.

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[6] Wheat 64.0
Casein 10.0
Skim milk powder 10.0
Salt mixture 3.6
Dextrin 7.4
Butter fat 5.0

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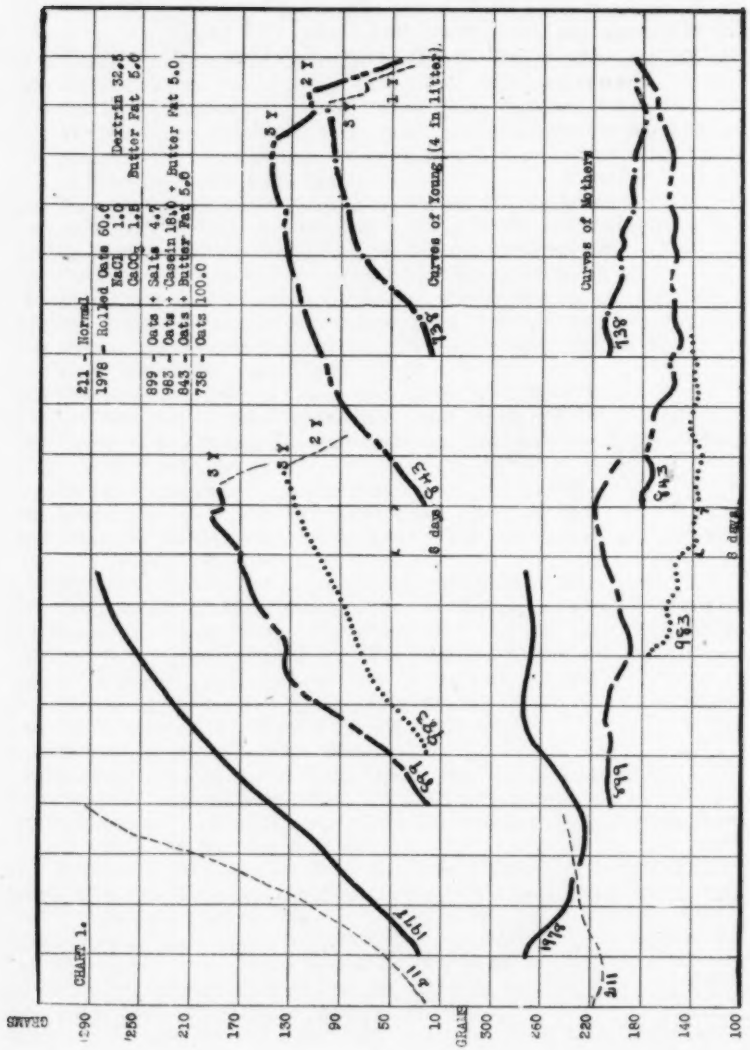

211 – Normal
1978 – Rolled Oats 60.0
NaCl 1.0
CaCO3 1.5
Dextrin 32.5
Butter Fat 5.0
899 – Oats + Salts 4.7
983 – Oats + Casein 18.0 + Butter Fat 5.0
843 – Oats + Butter Fat 5.0
736 – Oats 100.0
Curves of Young (4 in litter)
Curves of Mothers
GRAMS
GRAMS
CHART 1.
8 days
8 days

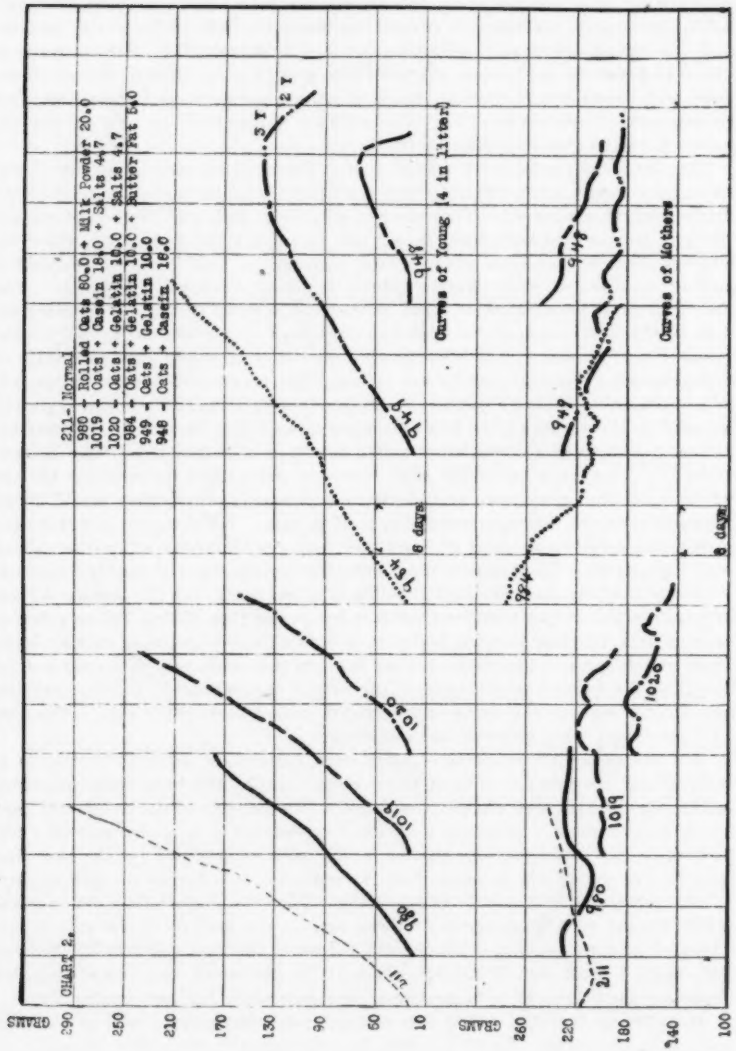
Chart 1. These curves illustrate the dietary value of the milk of a mother fed rolled oats alone, rat 738; oats plus fat-soluble A, rat 843; oats plus such salt additions as were necessary to correct the inorganic deficiencies of the oat kernel, rat 899; and oats plus salts plus fat-soluble A, rat 1978. For comparison there is drawn on each chart the excellent growth curve of four young whose mother received our 211 ration, which is one of excellent quality for inducing growth and reproduction (53). The weights represented by the curves are those of the four young collectively.

Rat 738: Diet rolled oats 100 per cent. These young grew decidedly slower than they should up to the 16th day after birth, and increased in weight very little from that time on. They died on the 37th, 40th and 44th days respectively. Rolled oats have been shown (12) to require the addition of three inorganic elements, sodium, chlorine and calcium, an addition of fat-soluble A and an addition of more protein before becoming dietetically complete. The fact that these young grew as much as they did is proof that the inorganic content of the milk was much more satisfactory than that of the oat kernel for the promotion of growth. Rat 899 was able on a diet of rolled oats plus salts to induce much greater growth in her young. The proteins of the oat kernel are of better quality than are those of wheat or maize as is shown by the good growth secured in the young of rat 1978 as compared with lots 1943 (chart 4) and 1942 (chart 6), whose diet consisted of rolled oats plus salts and butter fat (fat-soluble A). The young of rat 738 were, however, not normal for at about the age of 18 to 19 days they were seized with paroxysms in which they would throw themselves about the cage, screaming as if in pain. Within a few minutes they would fall exhausted. This performance they would repeat after intervals of varying lengths. They usually died soon after being observed in this condition.

Rat 843, whose diet consisted of rolled oats and butter fat (fat-soluble A) was not able to induce much better growth in her young than did rat 738 on a diet of oats alone. The first limiting factor in oats as a food for growing animals is the inorganic content of the seed, and the same is true with oats as a food for the lactating mother. The shortage of the factor, fat-soluble A, is also very important for without this being added growth ceased at an early date. The eyes of these young were inflamed but not swollen.

Rat 983 whose diet consisted of rolled oats, purified protein and fat-soluble A, was not able to produce milk of much better quality than she could havedone with either one of these additions omitted. The proteins of the oat do not need much improvement. Although additional fat-soluble A is needed and its addition distinctly improved the quality of the milk as is shown by the fact that growth was continuous at about half the optimum rate during the first 45 days of life, the young at that time began to die. That it was the butter-fat addition which caused such improvement as was seen in the quality of the milk is emphasized by a comparison of the growth curves of the young of rats 738, 843 and 983, chart 1, with that of rat 948, chart 2. In the latter case oats and purified protein (casein) proved to be no better than oats alone for milk production.

Rat 899, on a diet of rolled oats and salts did surprisingly well in inducing growth in her young. Up to the 24th day their growth was nearly normal. At this age young rats begin to eat of the mother's ration. Young rats do not grow at all when restricted to this diet (12). This shows that the mother can

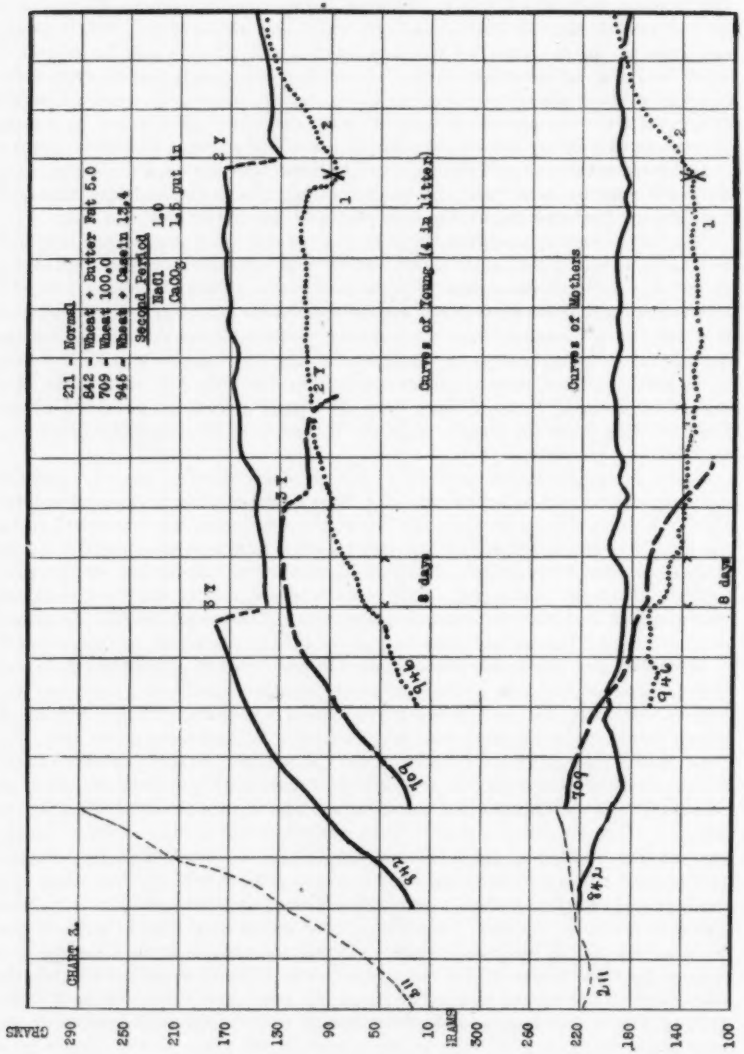

211- Normal
980 + Rolled Oats 80.0 + Milk Powder 20.0
1019 + Oats + Casein 18.0 + Salts 4.7
1020 + Oats + Gelatin 10.0 + Salts 4.7
984 + Oats + Gelatin 10.0 + Butterfat 5.0
949 - Oats + Gelatin 10.0
948 - Oats + Casein 18.0
CHART 2.
GRAMS
Curves of Young (4 in litter)
Curves of Mothers
3 Y
2 Y
8 days
8 days

take a diet, the mineral content of which is not suitable for growth in the young, and produce milk which has a distinctly better mineral content for the support of growth than had the food from which she produced it. Before young can grow at all on oats after weaning, both a salt mixture and fat-soluble A must be added. This ability to fare better than other body tissues when nourished by a blood stream which is too low in certain mineral elements to permit of symmetrical body growth, distinguishes the mammary gland from the muscle, nervous and glandular tissues and other tissues of the young. The fact that these young continued to grow between the 24th and 45th day indicates that they were still securing milk from the mother during this period and that this milk supplemented in some degree the deficiencies of the diet of oats and salts.

Rat 1978 whose diet contained but 60 per cent of rolled oats (9 per cent protein) supplemented with salts and fat-soluble A, was able to induce continuous growth at somewhat below the optimum rate over a period of 60 days. On this diet young rats cannot grow to any appreciable extent (12), (54). It follows from this that these young must have been getting such a milk supply from the mother during the entire sixty days as supplemented the oat and salt diet with respect to fat-soluble A and protein otherwise, judging from our earlier records, they should have ceased to grow when the milk supply gave out. It would appear from this that when the female rat is not fertilized she may continue to produce milk for at least sixty days.

Chart 2, completing the series of experiments described in chart 1. Rat 980, whose diet consisted of rolled oats plus 20 per cent of skim milk powder (Merrell-Soule) was able to induce good but not the optimum rate of growth in her young. It is possible that this was due to a low intake of fat-soluble A, rather than to failure of 20 per cent of skim milk powder to supplement the inorganic deficiencies of the oat kernel. This view is supported by the records of rats 1020 and 1019, in both of which oats plus protein and salts were fed and the growth of the young fell below normal because of the inadequate supply of fat-soluble A.

Rats 1020 and 1019 show, respectively, the good growth which the young were able to make on the milk produced by oats supplemented in the first case with gelatin and salts, and in the second by casein and salts. The growth of the young was near the optimum rate in both cases. In a previous paper (54) it has been shown that gelatin supplements the oat proteins better than does casein for the production of growth in the young. From the rate of development of the young on these two rations one would be inclined to pronounce both highly satisfactory, but we know, however, from the results of a long series of feeding experiments, that these diets were too poor in the dietary factor, fat-soluble A to promote optimum wellbeing over a prolonged period. Such studies have made it clear that in order to correctly evaluate any food mixture it is essential that not only "normal" curves of growth be secured, but the animals must be observed until it becomes apparent whether they breed at the same age as do rats on the best rations, and if later, how much. It must be determined whether they produce the normal number of young and rear them almost without exception to the weaning age, and observations should determine whether the second generation can develop satisfactorily on the diet. If the female rat is near the optimum in nutrition, she will become fertilized usually within two weeks after being freed from her nursing young and placed with a male. In ad-


211 – Normal
842 – Wheat + Butter Fat 5.0
709 – Wheat 100.0
946 – Wheat + Casein 13.4
Second Period
NaCl 1.0
$CaCO_3$ 1.5 put in
Curves of Young (4 in litter)
Curves of Mothers
8 days
GRAMS
GRAMS
CHART 3.

dition to these observations, valuable data can be secured by observing the age at which the animals first show signs of senility as shown by skin and coat changes and by general appearance. *Only with such observations can one say with finality what are the relative biological values of a series of rations.*

The curve of rat 984 presents the records of young which grew at half the normal rate on the milk derived from the mother's diet of rolled oats, gelatin and butter fat. On account of the low content of chlorine, sodium and calcium, this ration will not induce any growth in young animals. The mother was able to accumulate the necessary salts from the blood stream and to secrete a milk which contained a more favorable mineral content than did the food which she was taking. Attention has already been called to similar results with other diets with the same type of deficiency. (Rats 738, 843, 983, chart 1); (rats 949 and 948, chart 2).

Rat 949, whose ration consisted of oats plus gelatin, induced about half normal growth in her young but the young died between the 50th and 60th days. Oat proteins and gelatin constitute a mixture of high biological value for growth (54). The inorganic content of this diet is the first limiting factor as is shown by a comparison of the curves of the young of rat 949 with those of rat 1020. We have not observed good results in feeding growing rats on diets derived principally from rolled oats (12).

Rat 948 received a diet of rolled oats and casein. The result was the production of milk which was faulty in character (if we may safely compare the rat with the cow), and not so constituted as to enable young rats to grow when restricted to it as their sole food. The mortality of the young in the first week of life was 100 per cent for the other six litters which we observed. We have no explanation to offer for this result. (See table 1, chart 2.)

Chart 3. These curves show the dietary value of the milk of the mother rat when limited to whole wheat and to whole wheat supplemented with either purified protein or fat-soluble A. The curve of 211 represents the growth of a litter of four young whose mother was receiving a highly satisfactory diet, described in the legend to chart 1.

Rat 709, which was limited to whole wheat as her sole food, succeeded in producing milk which supported growth in her young during the first 24 days, after which they gained but very little. It is not known how long the young succeeded in extracting any milk from the mother. One young had already died on the 49th day and the remaining ones died on the 53rd and 57th days respectively. We have pointed out that young rats do not grow at all when confined to a diet of whole wheat (10). It follows, therefore, that the mother in this experiment was producing milk from a diet upon which the young were themselves unable to grow and the milk was capable of supporting growth at more than half the normal rate during the first 25 days. Either lactation fell off at this time or the quality of the milk greatly deteriorated as the stores of the mother's body became depleted. The latter explanation seems to harmonize best with the character of the curve of the mother and with the persistence with which other species, as the cow, are known to continue in lactation under conditions of faulty nutrition. On this ration of whole wheat alone, the eyes of both mother and young were inflamed and swollen, indicating partial starvation for the dietary factor fat-soluble A (5).

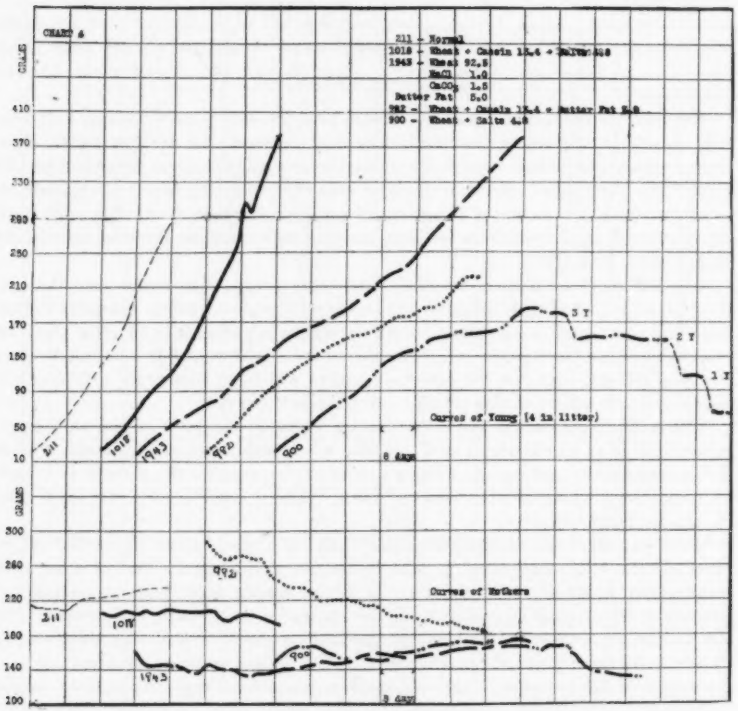


CHART 6
211 - Normal
1018 - Wheat + Casein 13.4 + Salts 4.8
1943 - Wheat 92.5
NaCl 1.0
CaCO3 1.5
Butter Fat 5.0
982 - Wheat + Casein 13.4 + Butter Fat 5.0
900 - Wheat + Salts 4.8
GRAMS
410
370
330
290
250
210
170
130
90
50
10
3 Y
2 Y
1 Y
Curves of Young (4 in litter)
8 days
GRAMS
300
260
220
180
140
100
Curves of Mothers
211
1018
1943
982
900
8 days

Rat 842 received a diet of wheat plus fat-soluble A and induced growth in her young at a nearly normal rate during the first ten days, after which the rate fell off somewhat. They were kept in steady growth during 45 days, however, by the milk of the mother when she was taking a diet which was itself incapable of supporting any growth whatever in the young. The most striking feature of this is in her ability to put into the milk an inorganic mixture of such composition and amount as is greatly superior to that contained in her food.

Rat 946 which was taking a diet of wheat and casein fell slightly below the performance of rat 709, on wheat alone. This is probably to be accounted for by the lowering of the inorganic intake of the mother through the dilution of the wheat with purified casein. The protective capacity of the mother in her relation to her young is easily seen, for on this food mixture young rats cannot grow at all (10) while the lactating mother can take it and produce milk with fair growth-promoting power. Her capacity to do this does not hold up efficiently beyond a limited period, as is to be expected. An addition of a salt mixture was made on the 86th day after the young were born. There was a prompt response with growth in the two remaining young and an increase in weight by the mother.

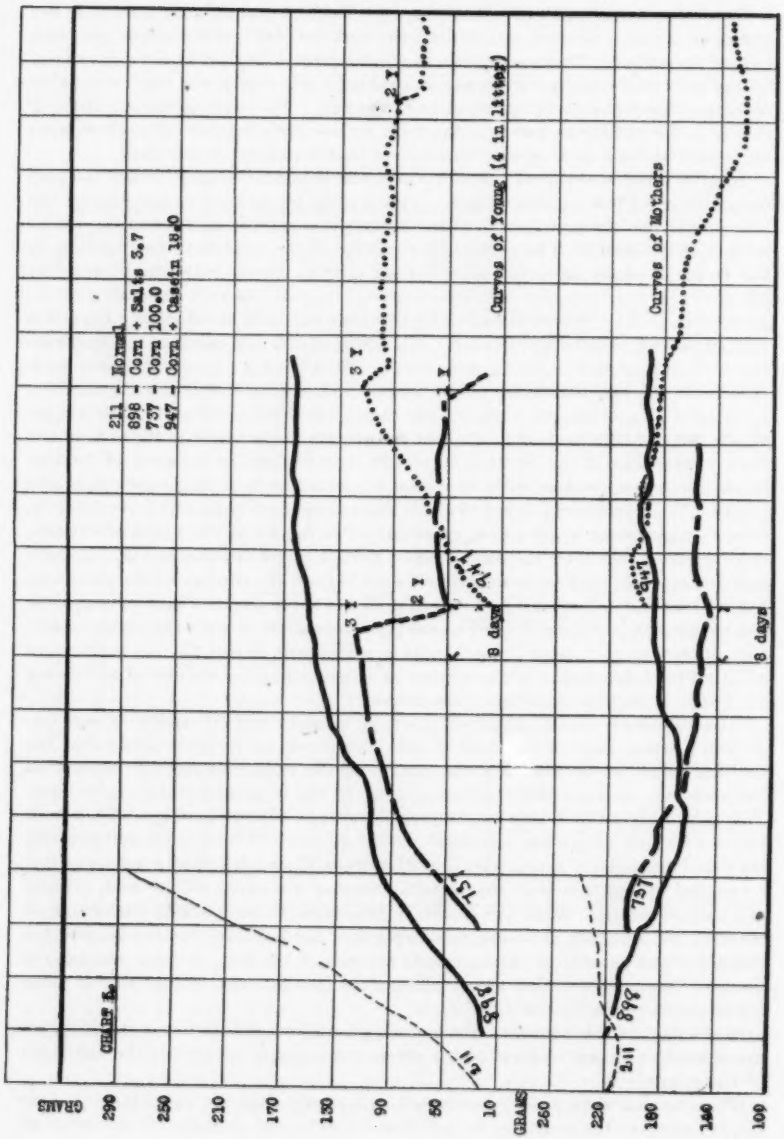
Chart 4. These records complete the series described in chart 3. The curves of rats 900 and 1943, both indicate that the quality of the wheat proteins is so low that it forms one of the limiting factors in determining the capacity of the lactating mother to produce milk of such a character as to promote growth in the young. The addition to wheat of either salts alone or of salts and fat-soluble A, fails to supplement whole wheat so as to enable her to produce milk of the best quality, as is shown by the shape of the growth curve for the young. Growth was continued in both these experiments far beyond the time at which the young begin to depend in large measure upon the diet of the mother. Since some growth can be secured in young rats fed wheat plus salts alone or with the further addition of butter fat (fat-soluble A) the records here shown do not indicate so great a protective action of the mother in safeguarding the welfare of her young as is seen in certain other cases described.

Rat 982 whose ration consisted of wheat, protein and fat-soluble A was unable to produce milk of excellent quality and there can be little doubt that the limiting factor in determining the quality of the milk was the low content of the elements, sodium, chlorine and calcium in the food mixture of the mother. This is strikingly illustrated by the growth curve of the young of rat 1018, whose ration of whole wheat was improved by the addition of both salts and protein. On this food mixture the mother was able to produce milk of excellent quality.

As just stated, rat 1018 succeeded in rearing young on wheat with protein and salt additions. When her record is considered in comparison with those of rats 900, 982 and 1943, it is seen how dependent the lactating mother is upon the character and amount of the inorganic content of her diet, and the comparison likewise demonstrates that she is much more independent of this factor than is the young rat in its ability to grow.

In the light of these records, the lactating mother stands in a new and hitherto unsuspected relation to diets which are in some degree faulty for the nutrition of the young.

Wheat is shown by these records to be decidedly richer in fat-soluble A, and the behavior of the young to be influenced less by the omission or inclusion of


211 - Normal
898 - Corn + Salts 3.7
737 - Corn 100.0
947 - Corn + Casein 18.0
Curves of Young (4 in Litter)
Curves of Mothers
8 days
8 days
GRAMS
GRAMS
CHART No.
3 Y
2 Y
1 Y
3 Y
2 Y
1 Y
947
737
898
211

this factor than was the case with oat diets. (Compare chart 2, lots 1019 to 1020.) The dietary properties of wheat are discussed in full in a former publication (10).

Chart 5. The curves shown in this chart illustrate the behavior of young rats when dependent upon the milk produced by mothers whose diets were restricted to the maize kernel and to maize with single purified food additions.

Rat 737 was restricted to a diet of ground corn. The growth of her young was only about half the normal rate but was continued for three weeks after the young are supposed normally to depend upon the adult diet. This means that the milk which the mother continued to contribute during the entire 45 days of the record served as an important factor of safety for the young. Two young died on the 42d and 45th days respectively, and the others were unable to develop further on a diet of corn plus such milk as the mother was able to contribute after that time.

Rats 898 and 947, whose diets consisted of corn plus salts and corn plus protein respectively, both failed to produce milk which had much growth-promoting power. As in the case of wheat, the two factors of greatest importance in making corn a poor food for milk production are the inorganic content and the poor quality of its proteins. This is made evident by the record of rat 1017, chart 6. She was able to induce nearly the optimum rate of growth in her young when the protein and inorganic contents of the corn kernel were supplemented by purified food additions. (See also chart 4, lot 1018.)

Chart 6. These records continue the series described in chart 5. Rat 844, whose diet consisted of corn and butter fat did no better in inducing growth in her young than did those in chart 5, where corn without any additions and with single additions other than fat-soluble A were fed. Both corn and wheat appear from the nursing records described as of distinctly better quality with respect to their content of fat-soluble A than is rolled oats. These records are in harmony with our studies on growth with these grains (10), (11), (12). The oat kernel contains proteins of distinctly better quality than are those of the wheat or corn kernels. This is shown by the fact that in the case of both wheat and corn both fat-soluble A and protein must be added before nearly normal milk could be produced, whereas in the case of the oat kernel, rat 1978 (chart 1), showed marked superiority in the quality of her milk as compared with rat 1943 (chart 4), whose ration consisted of wheat supplemented with salts and butter fat, and rat 1942 (chart 6), whose diet consisted of corn with the same supplements.

Rats 981 and 1942, when compared, demonstrate that for the production of normal milk the maize kernel must be improved with respect to both the protein and inorganic factors. The second periods in these two curves have little significance in the matter of showing anything about the quality of the milk which the mothers may have been producing at that time. It can, however, be readily seen from the growth of the young of rat 1017 (chart 6), that the quality of the milk produced is excellent when the diet of maize is supplemented with both protein and salts. In the second periods the young of rats 981 and 1942 should have been able to grow on the diets supplied them, without any aid in the way of supplemental milk feeding by the mother. These parts of the curves demonstrated clearly the capacity of the young to grow when the character of the diet was satisfactory.

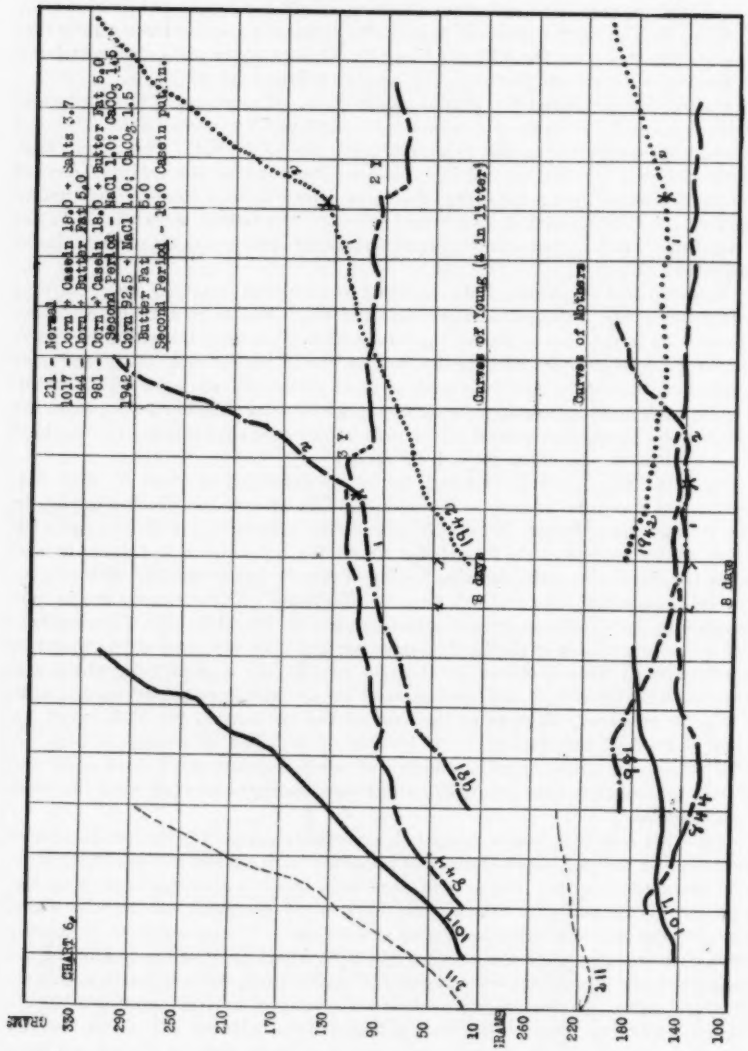


CHART 6
211 - Normal
1017 - Corn + Casein 18.0 + Salts 3.7
844 - Corn + Butter Fat 5.0
961 - Corn + Casein 18.0 + Butter Fat 5.0
Second Period - NaCl 1.0; $CaCO_3$ 1.5
1942 - Corn 92.5 + NaCl 1.0; $CaCO_3$ 1.5
Butter Fat 5.0
Second Period - 18.0 Casein put in.
Curves of Young (4 in litter)
Curves of Mothers
8 days
8 days
GRAMS
GRAMS
350
290
250
210
170
130
90
50
10
260
220
180
140
100

Fig. 1 Fig. 2

Fig. 1. The picture above shows the appearance of the normal eyes of the rat. The picture below shows the condition of the eyes which is brought on by a lack of sufficient fat-soluble A in the diet. This we have described as a type of xerophthalmia.

Fig. 2. The rat above was 78 days old and weighed 52 grams when photographed. The diet of its mother was satisfactory in all respects, except that the quality of the protein was poor. The protein of a diet derived solely from seeds and seed products is inadequate for the formation of normal milk.

The rat below was 59 days old and weighed 160 grams when photographed. The milk of the mother was of excellent quality and supported rapid growth in the young.

Both animals had access to the food of their mothers as soon as they were able to run about. At the age of 25 days the rat above weighed 16 grams, that below weighed 45 grams.

SHOCK AND CIRCULATORY FAILURE FOLLOWING TRAUMA

CARL J. WIGGERS

From the Physiological Laboratory, Cornell University Medical College, New York City

Received for publication April 20, 1918

I. INTRODUCTION

In spite of the general impression that trauma and severe pain frequently lead directly to shock, there is still a great diversity of opinion as to whether experimental shock can be produced by these agents alone. Occasionally nerve stimulation or crushing the testes is reported to have been successful in producing shock in experimental animals (1). Most experimenters, however, finding that prolonged stimulation of nerves or infliction of trauma does not reduce the mean blood pressure to a low level, give up this mode of experimentation and resort to such measures as exposing of the intestines, clamping the inferior vena cava, etc.

Although exposure and handling of the intestines probably offer the most certain means of inducing circulatory failure, attention has been frequently directed to the fact that such drastic procedures are rarely if ever reduplicated in man, even in the most extensive laparotomies. Furthermore, the extreme congestion of the splanchnic vessels found in these conditions is apparently rare in those human cases of traumatic and surgical shock that come to operation (2). Small wonder, therefore, that the question is frequently raised whether the circulatory conditions thus inaugurated are comparable to those found in other forms of shock.

It was therefore decided to reinvestigate the question whether pain and trauma can produce shock in experimental animals and, if successful, to compare the dynamics of the circulation in this condition with that following intestinal exposure.

II. THE CRITERION OF CENTRAL NERVOUS SYSTEM SHOCK AND CIRCULATORY FAILURE

The term "shock" is now commonly employed, especially among laboratory investigators, to characterize a condition of low arterial pressure and an impaired peripheral circulation; in short, the term is used synonymously with "circulatory failure." There is strong evidence, clinical, experimental and in some cases morphological, that functional or physical damage to the central nervous system forms an important part of the picture to which the term "shock" is clinically given (3). The degree of involvement is apparently variable, depending on the nature, intensity and location of the injury. In trauma involving the spinal cord or brain, complete loss of consciousness and all the phenomena characteristic of spinal shock produced in the laboratory may be present. If the injury is peripheral the sensibility may be reduced, a state of apathy exist and the reflexes may be abolished, or these signs of central nervous involvement may be less extreme and all of the reflex reactions and even the muscular power may be normal. The latter is apparently the case in "wound shock" (4). In regarding the shock producing power of trauma applied peripherally, therefore, it is necessary to consider not only whether the blood pressure may be reduced to a low level in this way but whether demonstrable functional changes in the central nervous system take place. In order to avoid confusion the term "central nervous system shock" is employed as descriptive of a condition in which functional impairment of the central nervous system, recognizable by such signs as apathy, reduced or abolished sensibility and loss of reflexes, is present. To the dynamic circulatory changes which lead to an impaired blood-supply of the tissues and low blood pressures the term "circulatory failure" is applied. Upon this basis separate criteria as to whether the animal is in a condition of central nervous system shock and whether circulatory failure is present must be established.

A criterion for determining the presence of "central nervous system shock" is readily formulated. Since normal dogs, even after prolonged ether anesthesia (six to eight hours) recover from ether, as given by us, in less than fifteen minutes, we may safely consider "shock" involving the central nervous system present when, after discontinuing the anesthetic, the animals lie in a relaxed state several hours and when during this interval they fail to react to sensory stimulation.

The degree of circulatory involvement is more difficult to estimate. Complete circulatory failure is obviously present when the mean arterial pressure is exceedingly low and certainly imminent when the venous pressure has fallen markedly. Evidence is accumulating, however, that a very serious involvement of the circulation may be present without a marked reduction of mean arterial pressure. Circulatory efficiency, as correctly emphasized by Henderson (5), depends primarily on the volume of blood perfusing the organs. Of this the pressure curve recorded by the mean pressure manometer is an even less accurate indicator than is commonly appreciated. To determine the volume flow through all the organs of the body directly is not a simple procedure and can not be attempted without materially disturbing the circulation. In many cases, however, the pressure curves of the arteries, when optically recorded, are of differential value. A normal flow through the peripheral organs predicates that a considerable flow must continue during the relatively long interval of diastole. When, therefore, the decline of pressure (which indicates the rate of volume flow through the peripheral vessels for the major portion of the cardiac cycle) occurs only during systole, while the pressure declines very slightly or not at all during diastole, we may be certain that marked circulatory involvement exists, no matter what the mercury manometer registers.

III. METHOD AND PROCEDURE

In order to study the question whether pain and trauma *per se* are able to induce shock, it is necessary, on the one hand, to adequately control all accessory or contributory influences arising in the experimental method and, on the other, to make certain that conditions for producing shock are approximately as favorable as in man.

Anesthesia. It seems probable that the potency of peripheral stimuli and trauma in producing reactions in the body leading to shock and circulatory failure depends, to a considerable extent, on how little the conducting mechanisms of the spinal cord and brain are depressed by the anesthetic. Forbes and Miller (6), using the action currents as a guide, found that deep ether anesthesia abolishes or, at least, greatly reduces nerve impulses to the cerebrum. The anesthesia, therefore, should be light and only sufficient to abolish pain sensation. It is possible indeed that failure to induce shock readily by traumatic influences may in part be due to too deep an anesthesia. According to

Henderson and Haggard (7), light ether anesthesia must be cautiously employed, however, for the possibility exists that the attendant stimulation of respiration itself may result in shock. To control this factor the ether anesthesia in about half of the experiments was preceded by a small dose of morphine and deep breathing thereby avoided. Two experiments were performed under morphine anesthesia alone after the preliminary operation had been completed. Etherization in all experiments was carried out as follows: A large amount of ether was poured into a cone tightly wrapped with towels and the animal rapidly anesthetized. Tracheotomy was then quickly performed and, during the rest of the operation, ether was administered through the trachea by the closed method. Having completed the operative procedures required for attaching manometers to record venous pressures, intrathoracic pressure variations, mean arterial pressure and the optical tracing desired, the trachea was connected by inspiratory and expiratory valves, the former in partial circuit with a Brodie ether bottle. From now on fresh air admixed with ether was supplied for each inspiration and the expired air escaped by the expiratory valve. Observations were then immediately started.

As in the research recently communicated (8), the gross features of the circulation were followed by continuous records and observation of the respiration and mean arterial pressure. The differential venous pressure was at first followed by a differential water manometer but, as this was found to be absolutely erratic, sometimes even as regards directional changes of effective venous pressures, during and following the very excessive breathing brought about in these experiments, it was discarded. Instead, the effective venous pressure was determined as in former years (9) by algebraic addition of the right auricular pressure (read directly on a water manometer) and the intrathoracic pressure variations recorded by a calibrated tambour on the drum. For this reason the readings of effective venous pressures here reported are absolute and not merely relative as in a paper published recently (8). After a preliminary half-hour's observation of the normal circulation the testes were crushed periodically or the sciatic nerve was dissected out and the central end stimulated for approximately two minute intervals between which a pause of thirty seconds was allowed.

IV. EFFECTS ON THE REACTIONS OF THE CENTRAL NERVOUS SYSTEM

Crushing of the testes and spermatic cord and stimulation of the sciatic nerves caused (with one exception in twenty-one experiments) a very intense effect both on the depth and rate of respirations. In no instance, even after intensely deep breathing had continued for the greater part of two hours, did permanent apnea or death from respiratory failure, such as reported by Henderson (10) after intense artificial respiration, take place. Following the deep breathing a very short period of apnea occurred or the respirations became temporarily shallow. Continued periodically for a time interval ranging from forty minutes to one and one-half hours, this left most of the animals in a state of "central nervous system shock." This occurred in sixteen out of the twenty-one experiments; it was impossible to bring the remainder to this condition. The percentage is sufficiently large to conclude that the symptoms of "central nervous system shock" may be induced in this way. More in detail, the animals lay quietly when the anesthetic had been removed and failed to react to auditory, touch, temperature or pain stimuli. In several animals, after more than an hour had elapsed a sciatic nerve was dissected out and clamped, with no reaction or objection on the part of the animal. During this stage the winking reflex was present, the pupils were dilated, lachrymation and salivary secretions were profuse.

Of the sixteen experiments in which this state of shock supervened the outcome was fatal in seven, death being due to accidental causes in two. The other five may be said to have died of "shock." All of these fatal cases followed crushing of the testes, while none of the animals in which the sciatic nerve alone was stimulated succumbed. The remaining nine animals eventually recovered. In these shock was produced by crushing the testes in two animals; by sciatic stimulation in two and by combined crushing and stimulation in five. The mode of recovery was interesting. In most experiments evidence of recovery became manifest within two to four hours. It began with an increase in rate and depth of respirations and, in some instances, some slowing of the heart. The animal then reacted more and more readily to painful stimuli applied to the forelegs and thorax but remained unresponsive to similar stimuli applied to the posterior portions of the body. Gradually these also were effective and finally the animal began to show signs of consciousness. This stage usually required about an hour and at that time it was obviously necessary to

discontinue observations on the state of the circulation. Gradually voluntary movements reappeared, first in the fore- and then in the hind-legs. The dog then sat up and walked about, responded to calling and in some cases ate or drank. Several dogs in which the trachea and neck operations were surgically repaired survived until the next day and ate at that time. They were then killed.

V. CIRCULATORY CHANGES IN SHOCK

Circulatory changes, of course, were inaugurated by each infliction of trauma and upon stimulation of the sciatic nerves. The nature of these reactions is well known to physiologists. Sciatic stimulation promptly produces an elevation of mean arterial and effective venous pressures. The latter is undoubtedly due largely to the mechanical effect of deep breathing aided, perhaps, by the reflex cardiac slowing. The rise of mean pressure is predominantly due to a reflex vasoconstriction. Crushing of the testes likewise increases the effective venous pressure through the mechanical influence of augmented breathing. The mean arterial pressure, however, lowers, owing largely to the reflex vasodilation.

Considering the central nervous system effects produced by sciatic stimulation and crushing experiments in the light of these early circulation disturbances, it is obvious that the most serious involvement of the central nervous system is produced only when the peripheral vessels are primarily dilated and a passive anemia of the central nervous system is present.

The same holds true as regards the degree of circulatory involvement of the five animals that failed to develop "central nervous system shock." Stimulation of the sciatic nerve was used exclusively in three cases and a combination of sciatic stimulation and crushing of testes was utilized in the other two. In these cases the circulatory changes upon cessation of the noxious influences were not affected to any marked extent.

The sixteen animals that developed reactions of the central nervous system characteristic of shock may be grouped as follows, according to their circulatory condition:

A. Five animals died in shock. Four of these showed circulatory changes closely resembling those described in the case of progressive and complete circulatory failure following intestinal exposure. One animal showed no marked alteration of the venous or arterial pressure

but toward the end the circulation rapidly failed from an acute cardiac insufficiency.

B. Two animals showing pronounced degree of "central nervous system shock" and a considerable reduction of venous and arterial pressure died from accidental causes. These must be left out of consideration.

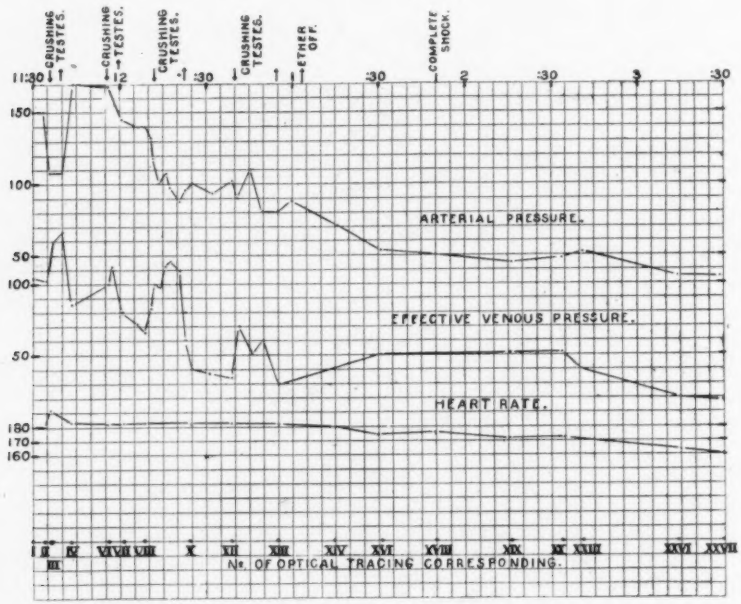


Fig. 1. Chart showing progressive changes in arterial pressure, effective venous pressure and heart rate following repeated crushing of testes and leading finally to death. Roman numerals refer to optical records of arterial pressure taken at corresponding points. Some of these are shown in figure 2.

C. Nine animals showed marked signs of "central nervous system shock" from which they subsequently recovered. All of these animals showed a distinct involvement of the circulation, although complete circulatory failure was at no time present. A more detailed analysis of the hemodynamics follows:

Shock accompanied by complete circulatory failure and terminating fatally. The gross changes of the circulation of one experiment are detailed in the plot

Fig. 2. Segments of pressure variations in central arteries taken at different stages of fatal circulatory failure by a calibrated optical manometer. Roman numerals correspond to points indicated on chart of figure 1.

of figure 1 and a few segments of the optical, arterial tracings corresponding in number to the points marked on the plot are shown in figure 2. For thirty minutes after the operation had been completed the mean arterial pressure averaged 150 mm. and the normal contour of the arterial pressure curve did not alter. Crushing of the testes was begun at 11.36 a.m. and was repeated at short intervals, as indicated by arrows on the plot of figure 1.

Every application of violence resulted in (1) a fall of mean arterial pressure; (2) a marked acceleration and augmentation of respiration with great expiratory effort; (3) an increase in the absolute and effective venous pressures. The latter occurred whether venous pressure was high or low, thereby demonstrating the mechanical ability of deep breathing to augment venous pressure. At first the fall of arterial pressure was accompanied by cardiac acceleration. The optical records obtained at this time show evidence of decreased arterial filling (fig. 2). The amplitude is greater, the primary wave larger and the systolic portion declines more rapidly (fig. 2, *II* and *III*). Inasmuch as the effective venous pressure actually increased during the stimulation and the heart accelerated, the diminished filling of the arterial trunks can be attributed only to a

Fig. 3. Three segments of optically recorded pressure variations from right ventricle taken from the case of shock summarized in table 1. Roman numerals correspond to those of this table.

reduction in peripheral resistance probably reflex in character. After cessation of a single (or sometimes several) attempt at crushing the testes, the respiration became slower and shallow, mean arterial pressure returned to a level above normal and the optical curves regained their normal contour (fig. 2, *IV* and *VI*).

Following frequent repetitions, however, the mean pressure recovered less and less and the effective venous pressure became progressively lower. The optical tracings (fig. 2, *VII* to *XIII*) show a progressive decrease in amplitude, indicating that the cardiac discharge was impaired. The heart became progressively slower. At 12.54 p.m. crushing was discontinued and the animal was left unmolested. Both the mean arterial and effective venous pressures continued on their downward course. Ether was discontinued at 1.03 p.m. and the animal never again reacted to painful stimuli until death occurred at 4.17 p.m. The optical curves are shown in segments *XVII*, *XVIII* and *XXVIII* of figure 2. Post mortem examination showed the intestinal loops to be pale and the veins poorly filled.

This experiment is typical of few cases in which trauma alone or

in combination with sciatic stimulation rapidly produced a condition of shock accompanied by circulatory failure recognizable by the fall of mean arterial and venous pressure alone. That the circulatory failure in these cases is similar to the cases of abdominal shock previously reported and predominantly of cardiac origin, is further suggested by the changing contour of the pressure curves optically recorded for the right ventricle. This is shown in an experiment from which the curves reported in figure 3 are taken. The tabular summary of this experiment is appended.

TABLE 1

Experiment C-165. January 25, 1918. Dog under ether anesthesia

TIME	BLOOD PRESSURE	EFFECTIVE VENOUS PRESSURE	HEART RATE	OPTICAL RECORD NUMBER	REMARKS
11.00		108	180	I	Ether started
12.15	128	110	170	III	
12.55	106	Higher			Crushing testes
12.56	120	120	202	IV	
1.03	112	120	165	V	
1.05					Ether off
1.07	116		160	VI	
1.09	136	130	170	VIII	
1.22					Ether on
1.57	120	80	216	X	After stimulating sciatic 15 minutes
2.07	116	133	216	XI	Sciatic stimulation
2.12	92	128			Sciatic stimulation stopped
2.27	106	60	260	XIV	Complete shock present
2.31	100	50	222	XV	
2.45	50	55	216	XVI	
3.03	40	35	202	XVII	
3.17	46	35	202	XVIII	
4.18	44	40	214	XIX	
4.50					Death

The circulatory dynamics in shock followed by recovery. Complete circulatory failure recognizable by a marked fall of effective venous or mean arterial pressure never occurred in any of the nine dogs that temporarily developed a complete state of "central nervous system shock" but recovered therefrom. In some instances, in fact, the arterial pressure was above 120 mm. during the state of shock. This indicates clearly that a low arterial pressure is not an essential condition of "central nervous system shock." The further conclusion that shock

may occur without circulatory involvement is not justified, however, for when carefully studied it is found that in this condition significant and often dangerous alterations of the circulation exist. These facts are illustrated in the following experiment:

TABLE 2

Experiment C-168. January 24, 1918. Anesthesia, light ether

TIME	BLOOD PRESSURE	EFFECTIVE VENOUS PRESSURE	HEART RATE	OPTICAL RECORD NUMBER	REMARKS
11.18	142	40	120		
11.25	140	40	120		
11.33	150	70	117	III	
11.38*	132	90	165	IV	After crushing testes
11.40	150	82	112	V	
11.44*	108	160			Crushing testes
11.48	151	66			
11.54*	126	160			Crushing testes
11.56*	160	130			Crushing testes continued
12.00*	140	120			Crushing testes stopped
12.05	128	70	160	VII	
12.13	132	72			
12.18*	150	120	230	IX	Crushing testes
12.35*	106	160			Crushing testes
12.39	118	120	230	XII	After crushing testes
12.50*	98	130	240	XIV	Crushing testes
1.00*	90	140			End of crushing
1.03	101	130	240	XVI	Animal in central nervous shock
1.40	126	102	165	XVII	
3.00	123	45	165	XIX	
3.35					Dog shows slight reaction from sensory stimuli; signs of recovery from shock
4.55					Animal conscious—reacts to sound

* Observations made during infliction of trauma.

During each crushing act the mean arterial pressure fell markedly and, owing largely to the mechanical result of the vigorous respiratory effects, the effective venous pressure was markedly elevated. If the mean pressures following the periods of relative rest between crushings alone are considered, the curve has a slightly downward course but never falls below 100. The corresponding curve of effective venous pressure gradually mounted, however, so that when the direct mechanical effect of the augmented breathing ceased (e.g., at 1.40 p.m.) the venous pressure still remained considerably elevated. Reduction of venous

Fig. 4. Segments of optically recorded arterial pressure changes from a case of "central nervous system shock" followed by recovery. Mean arterial pressure was sustained and effective venous pressure was elevated, as shown in tabular summary of table 2. Roman numerals correspond to those in this table.

pressure, therefore, scarcely played a rôle. It was true in six out of the nine cases which recovered that the effective venous pressure was moderately increased during the stage of shock. The heart progressively accelerated. A consideration of the optical tracings shows, however, that the arterial circulation was materially altered. This is shown in the segments of figure 4. A detailed analysis is scarcely necessary. The series of curves shows that, as in the initial stage characteristic of abdominal shock, the filling of the arterial trunks becomes progressively reduced. This reaches its climax at the end of the crushing period to which curve *XVI* corresponds. With each systole the blood column is thrown vigorously and the pressure drops more and more rapidly during the latter portion of systole. Following the incisura and throughout diastole the pressure is very low and declines but little, indicating that the peripheral flow is largely limited to systole. As the effective venous pressure is higher than before and the cardiac rate is increased, this can be accounted for only by a reduced peripheral resistance. From this condition recovery slowly sets in until forty minutes later when curves such as are shown in segment *XVII* are recorded and by the time the animal begins to react slightly to sensory stimulation

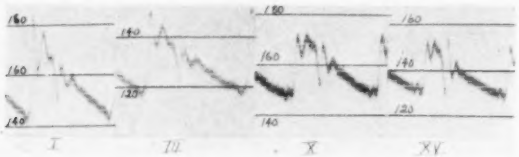


Fig. 5. Segments of optically recorded arterial pressure changes following prolonged sciatic stimulation and resulting in temporary central nervous system involvement. Details shown in table 3.

the normal characteristics again gradually begin to develop. In this case a bigeminal heart beat followed, as shown in segment *XIX*. This was clearly recognized by auscultation and disappeared an hour later. These cases show that whenever "central nervous system shock" develops as a result of trauma it is always accompanied by distinct circulatory changes in the nature of a prolonged low peripheral resistance. The mean arterial pressure is apparently maintained by the cardiac acceleration but, as is evident from the pressure curves of figure 4, is no longer a reliable index of the circulation.

Circulatory changes following prolonged sensory stimulation and resulting in temporary central nervous system shock. In two cases in which shock with subsequent recovery occurred as a result of prolonged sciatic stimulation, the mean arterial pressure was maintained at a level above the original but the effective venous pressure was gradually reduced to a moderate degree. The data from an illustrative experiment are shown in the following table:

TABLE 3

Experiment C-171. February 4, 1918. Anesthesia, ether without morphine

TIME	ARTERIAL PRESSURE	EFFECTIVE VENOUS PRESSURE	HEART RATE	OPTICAL RECORD NUMBER	REMARKS
11.53	145	74	174	I	
11.56–12					Crushing testes
12.02	116	78	146	III	After crushing testes
12.14	182	170			Stimulating sciatic
12.44	144	50	193	IV	After stimulation stopped
12.50	134	42	186	VI	
12.58	138	44	180	VII	
1.37	157	46	160	VIII	
1.42–1.55					
2.03	166	42	174	X	
2 27	152	40	174	XI	
2.31–2.39					Sciatic stimulation
2.40					Animal in shock
2.48	150	30	174	XIII	
3.18	140	38			
3.28	160	34	174	XIV	
3.34	154	34			
4.43	130	46	174		
4.50	146	46	174	XV	
5.20					Animal shows reaction to sensory stimulation
5.48					Conscious; struggles

The nature of the optical tracings corresponding to certain phases of the condition are shown in figure 5. It is evident that they are affected largely as regards amplitude, the pulse pressure decreasing and showing less essential changes in contour. The pressure at the beginning of diastole remains relatively high and the slope of the diastolic limb remains gradual. Contrary to the other cases, the lowered venous pressure and reduced cardiac discharge were compensated by an increased peripheral resistance. It seems that the pressor effect of stimulating the sciatic nerve becomes permanent in these cases, while the depressor effect of crushing the testes predominates in the others.

VI. CONCLUSIONS

1. A state of shock involving the central nervous system can be produced experimentally by trauma. This state may persist from two to five hours, after which recovery sets in; or it may be fatal.

2. Prolonged sensory stimulation may cause a temporary depression of the functions of the central nervous system but in itself does not lead to permanent changes or death.

3. "Central nervous system shock" never occurs without circulatory involvement which is always clearly indicated in optically recorded pressure curves from the arteries but is not necessarily evident in the mean pressure variations as given by the mercury manometer.

4. In the milder cases of shock; i.e., in those terminating in recovery, the circulatory derangement corresponds essentially to that described as characteristic in the initial and early progressive stage of circulatory failure in abdominal shock. Optical arterial pressure tracings show that a diminished volume of blood is contained in the arterial trunks and that the peripheral flow is thereby reduced. In most instances this is solely due to a reduction of the total arterial resistance while the effective venous pressure becomes somewhat increased through the mechanical effects of prolonged deep breathing. In a few cases only was the effective venous pressure reduced somewhat and constituted the main cause of arterial depletion.

5. In severe forms of shock; i.e., in those terminating fatally, the initial stage in which reduced peripheral resistance plays a rôle is of short duration, the effective venous pressure falls early, reaches a low level and by reducing the cardiac discharge, is the chief cause of complete circulatory failure.

6. The dynamic changes of the circulation which lead to progressive and complete circulatory failure are not essentially different in shock produced by trauma and that produced by intestinal exposure. The differences, if any, are in degree and duration of the respective phases but not in the character of the disturbance.

7. Considering all the available evidence, two factors may be said to be concerned in circulatory failure accompanying shock: *a*, the reduction of peripheral resistance; and *b*, the fall of effective venous pressure, decreasing the systolic discharge.

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AMERICAN JOURNAL OF PHYSIOLOGY

VOL. XLVI MAY 1, 1918 NO. 2

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